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(57) Abstract		<p>There is provided a novel vascular endothelial growth factor, herein designated VEGF-X, in addition to the nucleic acid molecule encoding it, a host cell transformed with said vector and compounds which inhibit or enhance angiogenesis. Also provided is the sequence of a CUB domain present in the sequence of VEGF-X which domain itself prevents angiogenesis and which is used to treat diseases associated with inappropriate vascularisation or angiogenesis.</p>																	
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- 1 -

VASCULAR ENDOTHELIAL GROWTH FACTOR-X

The present invention is concerned with a novel
vascular endothelial growth factor (VEGF) herein
5 designated "VEGF-X", and characterisation of the
nucleic acid and amino acid sequences of VEGF-X.

Introduction

10 Angiogenesis involves formation and proliferation of new blood vessels, and is an essential physiological process for normal growth and development of tissues in, for example, embryonic development, tissue regeneration and organ and tissue repair.

15 Angiogenesis also features in the growth of human cancers which require continuous stimulation of blood vessel growth. Abnormal angiogenesis is associated with other diseases such as rheumatoid arthritis psoriasis and diabetic retinopathy.

20 Capillary vessels consist of endothelial cells which carry the genetic information necessary to proliferate to form capillary networks. Angiogenic molecules which can initiate this process have previously been

25 characterised. A highly selective mitogen for vascular endothelial cells is vascular endothelial growth factor (VEGF) (Ferrara et al., "Vascular Endothelial Growth Factor: Basic Biology and Clinical Implications". Regulation of angiogenesis, by I.D.

30 Goldberg and E.M. Rosen 1997 Birkhauser Verlag Basle/Switzerland). VEGF is a potent vasoactive protein which is comprised of a glycosylated cationic 46-49 kd dimer having two 24 kd subunits. It is inactivated by sulphydryl reducing agents and is

35 resistant to acidic pH and to heating and binds to immobilised heparin.

- 2 -

VEGF-A has four different forms of 121, 165, 189 and 206 amino acids respectively due to alternative splicing. VEGF121 and VEGF165 are soluble and are capable of promoting angiogenesis, whereas VEGF189 and 5 VEGF206 are bound to heparin containing proteoglycans in the cell surface. The temporal and spatial expression of VEGF has been correlated with physiological proliferation of the blood vessels (Gajdusek, C.M., and Carbon, S.J., Cell Physiol., 10 139:570-579, (1989)); McNeil, P.L., Muthukrishnan, L., Warder, E., D'Amore, P.A., J. Cell. Biol., 109:811-822, (1989)). Its high affinity binding sites are localized only on endothelial cells in tissue sections (Jakeman, L.B., et al., Clin. Invest. 89:244-253 15 (1989)). The growth factor can be isolated from pituitary cells and several tumor cell lines, and has been implicated in some human gliomas (Plate, K.H. Nature 359:845-848, (1992)). The inhibition of VEGF function by anti-VEGF monoclonal antibodies was shown 20 to inhibit tumor growth in immune-deficient mice (Kim, K.J., Nature 362:841-844, (1993)).

VEGF proteins have been described in the following 25 patents and applications all of which are hereby incorporated by reference EP-0,506,477, WO-95/24473, WO-98/28621, WO-90/13649, EP-0,476,983, EP-0,550,296, WO-90/13649, WO-96/26736, WO-96/27007, WO-98/49300, WO-98/36075, WO-98/840124, WO-90/11084, WO-98/24811, WO-98/10071, WO-98/07832, WO-98/02543, WO-97/05250, 30 WO-91/02058, WO-96/39421, WO-96/39515, WO-98/16551.

The present inventors have now identified a further 35 vascular endothelial growth factor, designated herein as "VEGF-X", and the nucleic acid sequence encoding it, which has potentially significant benefits for the treatment of tumours and other conditions mediated by inappropriate angiogenic activity.

- 3 -

Summary of the Invention

In the present application, there is provided a novel vascular endothelial growth factor, herein designated 5 "VEGF-X", nucleic acid molecules encoding said growth factor, an expression vector comprising said nucleic acid molecule, a host cell transformed with said vector and compounds which inhibit or enhance angiogenesis. Also provided is the sequence of a CUB 10 domain present in the sequence of VEGF-X which domain itself prevents angiogenesis and which is used to treat diseases associated with inappropriate vascularisation or angiogenesis.

15 Detailed Description of the Invention

Therefore, according to a first aspect of the present invention there is provided a nucleic acid molecule encoding a VEGF-X protein or a functional equivalent, 20 fragment, derivative or bioprecursor thereof, said protein comprising the amino acid sequence from position 23 to 345 of the amino acid sequence illustrated in Figure 10. Alternatively, the nucleic acid molecule of the invention encodes the complete 25 sequence identified in Figure 10 and which advantageously includes a signal peptide to express said protein extracellularly. Preferably, the nucleic acid molecule is a DNA and even more preferably a cDNA molecule. Preferably, the nucleic acid molecule 30 comprises the nucleotide sequence from position 257 to 1291 of the nucleotide sequence illustrated in Figure 9. In a preferred embodiment the nucleic acid is of mammalian origin and even more preferably of human origin.

35

In accordance with the present invention a functional

- 4 -

equivalent should be taken to mean a protein, or a sequence of amino acids that have similar function to the VEGF-X protein of the invention.

- 5 Also provided by this aspect of the present invention
is a nucleic acid molecule such as an antisense
molecule capable of hybridising to the nucleic acid
molecules according to the invention under high
stringency conditions, which conditions would be well
10 known to those skilled in the art.

Stringency of hybridisation as used herein refers to conditions under which polynucleic acids are stable. The stability of hybrids is reflected in the melting
15 temperature (T_m) of the hybrids. T_m can be approximated by the formula:

$$81.5^{\circ}\text{C} + 16.6(\log_{10}[\text{Na}^+]) + 0.41 (\% \text{G\&C}) - 600/l$$

- 20 wherein l is the length of the hybrids in nucleotides. T_m decreases approximately by $1-1.5^{\circ}\text{C}$ with every 1% decrease in sequence homology.

25 The term "stringency" refers to the hybridisation conditions wherein a single-stranded nucleic acid joins with a complementary strand when the purine or pyrimidine bases therein pair with their corresponding base by hydrogen bonding. High stringency conditions favour homologous base pairing whereas low stringency
30 conditions favour non-homologous base pairing.

- 35 "Low stringency" conditions comprise, for example, a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate to low salt (SSC) concentration; or, alternatively, a temperature of about 50°C or less, and a moderate to high salt (SSPE) concentration, for example 1M NaCl.

- 5 -

"High stringency" conditions comprise, for example, a temperature of about 42°C or less, a formamide concentration of less than about 20%, and a low salt (SSC) concentration; or, alternatively, a temperature of about 65°C, or less, and a low salt (SSPE) concentration. For example, high stringency conditions comprise hybridization in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (Ausubel, F.M. et al. Current Protocols in Molecular Biology, Vol. I, 1989; Green Inc. New York, at 2.10.3).

"SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0.

"SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na₂HPO₄, and 1 mM EDTA, pH 7.4.

The nucleic acid capable of hybridising to nucleic acid molecules according to the invention will generally be at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the nucleotide sequences according to the invention.

The antisense molecule capable of hybridising to the nucleic acid according to the invention may be used as a probe or as a medicament or may be included in a pharmaceutical composition with a pharmaceutically acceptable carrier, diluent or excipient therefor.

The term "homologous" describes the relationship between different nucleic acid molecules or amino acid sequences wherein said sequences or molecules are related by partial identity or similarity at one or more blocks or regions within said molecules or

sequences.

The present invention also comprises within its scope
proteins or polypeptides encoded by the nucleic acid
5 molecules according to the invention or a functional
equivalent, derivative or bioprecursor thereof.

Therefore, according to a further aspect of the
present invention, there is provided a VEGF-X protein,
10 or a functional equivalent, derivative or bioprecursor
thereof, comprising an amino acid sequence from
position 23 to 345 of the sequence as illustrated in
Figure 10, or alternatively which amino acid sequence
comprises the complete sequence of Figure 10. A
15 further aspect of the invention comprises a VEGF-X
protein, or a functional equivalent, derivative or
bioprecursor thereof, encoded by a nucleic acid
molecule according to the invention. Preferably, the
VEGF-X protein encoded by said nucleic acid molecule
20 comprises the sequence from position 23 to 345 of the
amino acid sequence as illustrated in Figure 10, or
which sequence alternatively comprises the sequence of
amino acids of Figure 10.

25 The DNA molecules according to the invention may,
advantageously, be included in a suitable expression
vector to express VEGF-X encoded therefrom in a
suitable host. Incorporation of cloned DNA into a
suitable expression vector for subsequent
30 transformation of said cell and subsequent selection
of the transformed cells is well known to those
skilled in the art as provided in Sambrook et al.
(1989), molecular cloning, a laboratory manual, Cold
Spring Harbour Laboratory Press.

35 An expression vector according to the invention
includes a vector having a nucleic acid according to

- 7 -

the invention operably linked to regulatory sequences, such as promoter regions, that are capable of effecting expression of said DNA fragments. The term "operably linked" refers to a juxta position wherein 5 the components described are in a relationship permitting them to function in their intended manner. Such vectors may be transformed into a suitable host cell to provide for expression of a polypeptide according to the invention. Thus, in a further 10 aspect, the invention provides a process for preparing polypeptides according to the invention which comprises cultivating a host cell, transformed or transfected with an expression vector as described above under conditions to provide for expression by 15 the vector of a coding sequence encoding the polypeptides, and recovering the expressed polypeptides.

The vectors may be, for example, plasmid, virus or 20 phage vectors provided with an origin of replication, and optionally a promoter for the expression of said nucleotide and optionally a regulator of the promoter.

The vectors may contain one or more selectable 25 markers, such as, for example, ampicillin resistance.

Regulatory elements required for expression include 30 promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for translation initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or 35 homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the

- 8 -

ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art.

5 Nucleic acid molecules according to the invention may be inserted into the vectors described in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense nucleic acids may be produced by synthetic means.

10 In accordance with the present invention, a defined nucleic acid includes not only the identical nucleic acid but also any minor base variations including in particular, substitutions in cases which result in a 15 synonymous codon (a different codon specifying the same amino acid residue) due to the degenerate code in conservative amino acid substitutions. The term "nucleic acid sequence" also includes the complementary sequence to any single stranded sequence 20 given regarding base variations.

The present invention also advantageously provides nucleic acid sequences of at least approximately 10 contiguous nucleotides of a nucleic acid according to 25 the invention and preferably from 10 to 50 nucleotides even more preferably, the nucleic acid sequence comprise the sequences illustrated in Figure 3. These sequences may, advantageously be used as probes or primers to initiate replication, or the like. Such 30 nucleic acid sequences may be produced according to techniques well known in the art, such as by recombinant or synthetic means. They may also be used in diagnostic kits or the like for detecting the presence of a nucleic acid according to the invention. 35 These tests generally comprise contacting the probe with the sample under hybridising conditions and detecting for the presence of any duplex or triplex

- 9 -

formation between the probe and any nucleic acid in the sample.

5 The nucleic acid sequences according to this aspect of the present invention comprise the sequences of nucleotides illustrated in Figures 3 and 5.

10 According to the present invention these probes may be anchored to a solid support. Preferably, they are present on an array so that multiple probes can simultaneously hybridize to a single biological sample. The probes can be spotted onto the array or synthesised *in situ* on the array. (See Lockhart et al., *Nature Biotechnology*, vol. 14, December 1996 15 "Expression monitoring by hybridisation to high density oligonucleotide arrays". A single array can contain more than 100, 500 or even 1,000 different probes in discrete locations.

20 The nucleic acid sequences, according to the invention may be produced using such recombinant or synthetic means, such as for example using PCR cloning mechanisms which generally involve making a pair of primers, which may be from approximately 10 to 50 25 nucleotides to a region of the gene which is desired to be cloned, bringing the primers into contact with mRNA, cDNA, or genomic DNA from a human cell, performing a polymerase chain reaction under conditions which brings about amplification of the 30 desired region, isolating the amplified region or fragment and recovering the amplified DNA. Generally, such techniques are well known in the art, such as described in Sambrook et al. (*Molecular Cloning: a Laboratory Manual*, 1989).

35 The nucleic acids or oligonucleotides according to the invention may carry a revealing label. Suitable

- 10 -

labels include radioisotopes such as ^{32}P or ^{35}S , enzyme labels or other protein labels such as biotin or fluorescent markers. Such labels may be added to the nucleic acids or oligonucleotides of the invention and
5 may be detected using known techniques *per se*.

Advantageously, human allelic variants or polymorphisms of the DNA molecule according to the invention may be identified by, for example, probing
10 cDNA or genomic libraries from a range of individuals, for example, from different populations. Furthermore, nucleic acids and probes according to the invention may be used to sequence genomic DNA from patients using techniques well known in the art, such as the
15 Sanger Dideoxy chain termination method, which may, advantageously, ascertain any predisposition of a patient to certain disorders associated with a growth factor according to the invention.

20 The protein according to the invention includes all possible amino acid variants encoded by the nucleic acid molecule according to the invention including a polypeptide encoded by said molecule and having conservative amino acid changes. Conservative amino
25 acid substitution refers to a replacement of one or more amino acids in a protein as identified in Table 1. Proteins or polypeptides according to the invention further include variants of such sequences, including naturally occurring allelic variants which are
30 substantially homologous to said proteins or polypeptides. In this context, substantial homology is regarded as a sequence which has at least 70%, preferably 80 or 90% and preferably 95% amino acid homology with the proteins or polypeptides encoded by
35 the nucleic acid molecules according to the invention. The protein according to the invention may be recombinant, synthetic or naturally occurring, but is

- 11 -

preferably recombinant.

The nucleic acid or protein according to the invention may be used as a medicament or in the preparation of a medicament for treating cancer or other diseases or conditions associated with expression of VEGF-X protein.

Advantageously, the nucleic acid molecule or the protein according to the invention may be provided in a pharmaceutical composition together with a pharmacologically acceptable carrier, diluent or excipient therefor.

The present invention is further directed to inhibiting VEGF-X *in vivo* by the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation of antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion or the mature DNA sequence, which encodes for the protein of the present invention, is used to design an antisense RNA oligonucleotide of from 10 to 50 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple-helix - see Lee et al. *Nucl. Acids Res.*, 6:3073 (1979); Cooney et al., *Science*, 241:456 (1988); and Dervan et al., *Science*, 251: 1360 (1991), thereby preventing transcription and the production of VEGF-X. The antisense RNA oligonucleotide hybridises to the mRNA *in vivo* and blocks translation of an mRNA molecule into the VEGF-X protein (antisense - Okano, *J. Neurochem.*, 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)).

Alternatively, the oligonucleotide described above can be delivered to cells by procedures in the art such that the anti-sense RNA and DNA may be expressed *in vivo* to inhibit production of VEGF-X in the manner 5 described above.

Antisense constructs to VEGF-X, therefore, may inhibit the angiogenic activity of VEGF-X and prevent the further growth of or even regress solid tumours, since 10 angiogenesis and neovascularization are essential steps in solid tumour growth. These antisense constructs may also be used to treat rheumatoid arthritis, psoriasis and diabetic retinopathy which are all characterized by abnormal angiogenesis.

15 A further aspect of the invention provides a host cell or organism, transformed or transfected with an expression vector according to the invention. The host cell or organism may advantageously be used in a method of producing VEGF-X, which comprises recovering 20 any expressed VEGF-X from the host or organism transformed or transfected with the expression vector.

According to a further aspect of the invention there 25 is also provided a transgenic cell, tissue or organism comprising a transgene capable of expressing VEGF-X protein according to the invention. The term "transgene capable of expression" as used herein means a suitable nucleic acid sequence which leads to 30 expression of VEGF-X or proteins having the same function and/or activity. The transgene, may include, for example, genomic nucleic acid isolated from human cells or synthetic nucleic acid, including DNA integrated into the genome or in an extrachromosomal state. Preferably, the transgene comprises the 35 nucleic acid sequence encoding the proteins according to the invention as described herein, or a functional

- 13 -

- fragment of said nucleic acid. A functional fragment of said nucleic acid should be taken to mean a fragment of the gene comprising said nucleic acid coding for the proteins according to the invention or 5 a functional equivalent, derivative or a non-functional derivative such as a dominant negative mutant, or bioprecursor of said proteins. For example, it would be readily apparent to persons skilled in the art that nucleotide substitutions or 10 deletions may be used using routine techniques, which do not affect the protein sequence encoded by said nucleic acid, or which encode a functional protein according to the invention.
- 15 VEGF-X protein expressed by said transgenic cell, tissue or organism or a functional equivalent or bioprecursor of said protein also forms part of the present invention.
- 20 Antibodies to the protein or polypeptide of the present invention may, advantageously, be prepared by techniques which are known in the art. For example, polyclonal antibodies may be prepared by inoculating a host animal, such as a mouse or rabbit, with the 25 polypeptide according to the invention or an epitope thereof and recovering immune serum. Monoclonal antibodies may be prepared according to known techniques such as described by Kohler R. and Milstein C., Nature (1975) 256, 495-497. Advantageously, such 30 antibodies may be included in a kit for identifying VEGF-X in a sample, together with means for contacting the antibody with the sample.
- 35 Advantageously, the antibody according to the invention may also be used as a medicament or in the preparation of a medicament for treating tumours or other diseases associated with expression of VEGF-X.

- 14 -

The invention also further provides a pharmaceutical composition comprising said antibody together with a pharmaceutically acceptable carrier diluent or excipient therefor.

5

Proteins which interact with the polypeptide of the invention may be identified by investigating protein-interactions using the two-hybrid vector system first proposed by Chien et al., (1991) Proc. Natl. Acad. Sci. USA 88 : 9578-9582.

This technique is based on functional reconstitution in vivo of a transcription factor which activates a reporter gene. More particularly the technique comprises providing an appropriate host cell with a DNA construct comprising a reporter gene under the control of a promoter regulated by a transcription factor having a DNA binding domain and an activating domain, expressing in the host cell a first hybrid DNA sequence encoding a first fusion of a fragment or all of a nucleic acid sequence according to the invention and either said DNA binding domain or said activating domain of the transcription factor, expressing in the host at least one second hybrid DNA sequence, such as a library or the like, encoding putative binding proteins to be investigated together with the DNA binding or activating domain of the transcription factor which is not incorporated in the first fusion; detecting any binding of the proteins to be investigated with a protein according to the invention by detecting for the presence of any reporter gene product in the host cell; optionally isolating second hybrid DNA sequences encoding the binding protein.

35 An example of such a technique utilises the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate

- 15 -

domain for binding to activators upstream of the galactose metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide residues encoding the DNA binding domain of GAL4.

5 These binding domain residues may be fused to a known protein encoding sequence, such as for example, the nucleic acids according to the invention. The other vector comprises the residues encoding the protein binding domain of GAL4. These residues are fused to residues encoding a test protein. Any interaction between polypeptides encoded by the nucleic acid according to the invention and the protein to be tested leads to transcriptional activation of a

10 reporter molecule in a GAL-4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as β -galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes.

15

20 A further aspect of the present invention also provides a method of identifying VEGF-X in a sample, which method comprises contacting said sample with an antibody according to the invention and monitoring for any binding of any proteins to said antibody. A kit for identifying the presence of VEGF-X in a sample is also provided comprising an antibody according to the invention and means for contacting said antibody with said sample.

25

30 VEGF-X may be recovered and purified from recombinant cell cultures by methods known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography,

35 phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin

- 16 -

chromatography.

The VEGF-X protein of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated with mammalian or other eukaryotic carbohydrates or may be non-glycosylated.

VEGF-X is particularly advantageous as a wound healing agent, where, for example, it is necessary to re-vascularize damaged tissues, or where new capillary angiogenesis is important. Accordingly, VEGF-X may be used for treatment of various types of wounds such as for example, dermal ulcers, including pressure sores, venous ulcers, and diabetic ulcers. In addition, it can be used in the treatment of full-thickness burns and injuries where angiogenesis is desired to prepare the burn in injured sites for a skin graft and flap. In this case, VEGF-X or the nucleic acid encoding it may be applied directly to the wound. VEGF-X may be used in plastic surgery when reconstruction is required following a burn, other trauma, or even for cosmetic purposes.

An important application of VEGF-X is to induce the growth of damaged bone, periodontium or ligament tissue. For example, it may be used in periodontal disease where VEGF-X is applied to the roots of the diseased teeth, leading to the formation of new bone and cementum with collagen fibre ingrowths. It can be used for regenerating supporting tissues of teeth, including alveolar bone, cementum and periodontal

- 17 -

ligament, that have been damaged by disease and trauma.

Since angiogenesis is important in keeping wounds
5 clean and non-infected, VEGF-X may be used in association with surgery and following the repair of cuts. It should be particularly useful in the treatment of abdominal wounds where there is a high risk of infection.

10 VEGF-X can also be used for the promotion of endothelialization in vascular graft surgery. In the case of vascular grafts using either transplanted or synthetic material, VEGF-X may be applied to the
15 surface of the graft or at the junction to promote the growth of the vascular endothelial cells. One derivation of this is that VEGF-X can be used to repair the damage of myocardial and other occasions where coronary bypass surgery is needed by stimulating
20 the growth of the transplanted tissue. Related to this is the use of VEGFX to repair the cardiac vascular system after ischemia.

25 The protein of the present invention may also be employed in accordance with the present invention by expression of such protein *in vivo*, which is often referred to as "gene therapy".

30 Thus, for example, cells such as bone marrow cells may be engineered with a polynucleotide (DNA or RNA) encoding for the protein *ex vivo* as defined herein, the engineered cells are then provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be
35 engineered by procedures known in the art by use of a retroviral particle containing RNA encoding for the protein of the present invention.

- 18 -

Similarly, cells may be engineered *in vivo* for expression of the protein *in vivo*, for example, by procedures known in the art.

5 A further aspect of the invention comprises a method of treating a disorder mediated by expression of a protein according to the invention, by administering to a patient an amount of an antisense molecule as described herein, in sufficient concentration to
10 alleviate or reduce the symptoms of said disorder.

Compounds which inhibit or enhance angiogenesis may be identified by providing a host cell or organism according to the invention or a transgenic cell,
15 tissue or organism according to the invention, contacting a test compound with said cell, tissue or organism and monitoring for the effect of said compound compared to a cell tissue or organism which has not been contacted with said compound. These
20 compounds may themselves be used as a medicament or included in a pharmaceutical composition for treatment of disorders mediated by inappropriate vascularisation or angiogenic activity.

25 The present inventors have also, advantageously, identified in the sequence encoding the VEGF-X protein a CUB domain, which has heretofore not previously been identified in VEGF-type growth factors. The VEGF-X protein may therefore exert dual regulatory effects
30 via interaction with the VEGF tyrosine kinase receptors or with neuropilin receptors mediated by the CUB domain. Thus, the sequence encoding said CUB domain may be included in an expression vector for subsequent transformation of a host cell, tissue or
35 organism.

VEGF-X or fragments thereof may be able to modulate

- 19 -

the effects of pro-angiogenic growth factors such as VEGF as indicated in the findings presented in the examples below that the N-terminal part of the VEGF-X protein, a CUB-like domain, is able to inhibit VEGF-
5 stimulated proliferation of HUVECs. VEGF-X or fragments thereof may therefore be useful in therapy of conditions involving inappropriate angiogenesis. Inhibition of the angiogenic activity of VEGF has been linked with inhibition of tumour growth in
10 several models eg Kim K. J. et al, Nature 362:841-844, (1993). Additionally, agents able to inhibit angiogenesis would be expected to be useful in treating other angiogenesis-dependent diseases such a
15 retinopathy, osteoarthritis and psoriasis(Folkman, J., Nature Medicine 1:27-31, (1995).

As identified in more detail in the Examples described herein the present inventors have surprisingly identified that the CUB domain of VEGF-X
20 is able to inhibit stimulation of proliferation of HUVECs induced by either VEGF or bFGF. The CUB domain may, therefore, be utilised as a therapeutic agent for inhibition of angiogenesis and for treatment of condition associated with inappropriate
25 vascularisation or angiogenesis.

Therefore according to a further aspect of the invention there is provided a method of inhibiting angiogenic activity and inappropriate vascularisation including formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ and tissue repair in a subject said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 or a nucleic acid molecule encoding the CUB domain according to the invention in
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- 20 -

sufficient concentration to reduce or prevent said angiogenic activity.

Furthermore there is also provided a method of
5 treating or preventing any of cancer, rheumatoid arthritis, psoriasis and diabetic retinopathy, said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated
10 in Figure 10 or a nucleic acid molecule encoding the CUB domain according to the invention in sufficient concentration to treat or prevent said disorders.

The CUB domain may also be used to identify compounds
15 that inhibit or enhance angiogenic activity such as inappropriate vascularisation, in a method comprising contacting a cell expressing a VEGF receptor and/or a neuropilin 1 or 2 type receptor with said compound in the presence of a VEGF-X protein according to the
20 invention and monitoring for the effect of said compound or said cell when compared to a cell which has not been contacted with said compound. Such compounds may then be used as appropriate to prevent or inhibit angiogenic activity to treat the disorders
25 or conditions described herein, or in a pharmaceutical composition. An antibody to said CUB domain may also be useful in identifying other proteins having said sequences.

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- 21 -

Deposited Plasmids

		<u>Date of Deposit</u>	<u>Accession No.</u>
	Plasmid VEGFX/pCR2.1		
5	1TOPO FL	1 March 1999	LMBP 3925
	Plasmid VEGFX/pRSETB BD		
	amino acids	1 March 1999	LMBP 3926
10	G230-G345		
	Plasmid VEGFX/pcR.2.1		
	FL Clone 9	20 October 1999	LMBP 3977
15	Plasmid VEGF-X CUB		
	PET22b	20 December 1999	-----
20	The above plasmids were deposited at the Belgian Coordinated Collections of Microorganisms (BCCM) at Laboratorium Voor Moleculaire Biologie- Plasmidencollectie (LMBP) B-9000, Ghent, Belgium, in accordance with the provisions of the Budapest Treaty of 28 April 1977.		
25	The invention may be more clearly understood with reference to the accompanying example, which is purely exemplary, with reference to the accompanying drawings, wherein:		
30	Figure 1: is a DNA sequence identified in the Incyte LifeSeq™ database coding for a novel VEGF-X protein.		
35	Figure 2: is an illustration of amino acid sequence of the nucleic acid sequence of Figure 1.		

- 22 -

- 5 Figure 3: is an illustration of PCR primer sequences utilised to identify the VEGF-X protein according to the invention.

10 Figure 4: is a diagrammatic illustration of the spatial relationships in the VEGF-X sequence of the clones identified using the PCR primer sequences of Figure 3.

15 Figure 5: is an illustration of the nucleotide sequences of the 5' RACE primers used to identify the 5' end of the VEGF-X open reading frame.

20 Figure 6: is an illustration of the sequence obtained from the RACE experiment.

25 Figure 7: is an illustration of the nucleotide sequences obtained from the search of LifeSeq™ database using the sequence in Figure 6.

30 Figure 8: is an illustration of the primers used to clone the entire coding sequence of VEGF-X.

35 Figure 9: is an illustration of the entire coding sequence of VEGF-X.

40 Figure 10: is an illustration of the predicted amino acid sequence of the nucleotide sequence of Figure 9.

45 Figure 11: is an alignment of the sequence of

- 23 -

Figure 10 with the sequences of VEGF-A
to D.

5 Figure 12: is an illustration of variant
 sequences of the VEGF-X protein
 according to the invention.

10 Figure 13: is an illustration of the
 oligonucleotide primers used for
 E.coli expression of VEGF-X domains
 and for expression of the full length
 sequence of VEGF-X in a
 baculovirus/insect cell expression
 system.

15 Figure 14: depicts nucleic acid sequences of 18
 human EST clones obtained from a BLAST
 search of the LifeSeq™ database used
 to identify the full sequence encoding
 VEGF-X.

20 Figure 15: depicts the nucleotide sequences of 50
 human EST clones obtained from the
 LifeSeq™ database.

25 Figure 16: is an illustration of nucleotide
 sequences utilised as primers to
 identify the nucleotide sequence
 encoding VEGF-X.

30 Figure 17: is a nucleotide sequence coding for a
 partial VEGF-X protein according to
 the invention.

35 Figure 18: is an illustration of a partial
 nucleotide sequence encoding VEGF-X
 protein according to the invention.

- 24 -

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Figure 19: is an illustration of a DNA and polypeptide sequence used for mammalian cell expression of VEGF-X. The predicted VEGF-X signal sequence is in lower case letters. The C-terminal V5 epitope and His6 sequences are underlined.

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Figure 20: is an illustration of a DNA and polypeptide sequence used for baculovirus/insect cell expression of VEGF-X. In the polypeptide sequence the signal sequence is shown in lower case. The N-terminal peptide tag added to the predicted mature VEGF-X sequence is underlined.

20

Figure 21: is an illustration of a DNA and polypeptide sequence used for *E. coli* expression of VEGF-X. The polypeptide sequences at the N- and C- termini derived from the MBP fusion and His6 tag respectively are underlined.

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Figure 22: illustrates the disulphide-linked dimerisation of VEGF-X. Protein samples were analysed by SDS-PAGE. Prior to loading the gel, samples were heated to 95°C for 5 minutes in sample buffer in the presence (+) or absence (-) of reducing agent. (A) samples from COS cell expression of a C-terminally V5/His6 peptide-tagged construct. The left hand panel is total conditioned medium, the right hand panel is material purified on Nickel agarose resin. Reduced monomer

- 25 -

5 and putative disulphide-linked, non-reduced dimer are indicated by arrows.
There appears to be proteolysis of the
protein during purification. Gels were
blotted onto nylon membranes and
protein detected with an anti V5
monoclonal antibody. (B) Samples from
E.coli expression of a maltose-binding
protein/His6 dual fusion construct. M
10 indicates the molecular weight markers
(Benchmark, LifeTechnologies). The
gel was stained with Coomassie Blue by
standard procedures. The fusion
protein has an apparent molecular
15 weight of 80kDa.

Figure 23: 20 illustrates the glycosylation of VEGF-X. VEGF-X was purified from the culture supernatant of COS cells transfected with the pcDNA6/V5-His construct. Supernatants were harvested 72h post-transfection and purified on nickel resin. Samples were then treated with EndoH (+) or untreated (-) before SDS-PAGE and blotting, as described in the legend to Figure 22.

Figure 24: 30 is an illustration of the DNA and polypeptide sequence used for *E. coli* expression of the VEGF-like domain of VEGF-X. Polypeptide sequences at the N-terminus of the protein derived from the vector are underlined.

Figure 25: 35 shows expression of the VEGF-X VEGF domain in *E. coli*. Lane 1-10 μ l broad

- 26 -

range marker (New England Biolabs),
lane 2-10 μ l unreduced sample, lane 3-
10 μ l reduced sample. The reduced PDGF
domain protein (lane 3) has an
apparent molecular weight of
approximately 19kDa on SDS-PAGE.

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Figure 26:

illustrates a DNA and polypeptide sequence used for *E. coli* expression of the CUB-like domain of VEGF-X. The polypeptide sequence at the N-terminus derived from the vector-encoded signal and the introduced His6 tag are underlined.

Figure 27:

shows expression of the VEGF-X CUB domain in *E. coli*. The CUB domain protein was purified on Nickel chelate resin. The protein migrates at approximately 23kDa on SDS-PAGE.

Figure 28:

illustrates the effect of truncated VEGF-X (CUB domain) on HUVEC proliferation. (A) Human Umbilical Vein Endothelial Cells (one-day-treatment). (B) Human Umbilical Vein Endothelial Cells (24-hour starving followed by one-day-treatment). (C) Effect of VEGF-A₁₆₅ and VEGF-X CUB domain on the proliferation of HUVEC (two-day-treatment).

Figure 29:

depicts the tissue distribution of VEGF-X mRNA analysed by Northern blotting and RT-PCR in (A) normal tissues and (B) tumour tissue and cell lines.

- 27 -

- Figure 30: depicts the partial intron/exon structure of the VEGF-X gene. (A) Genomic DNA sequences of 2 exons determined by sequencing; exon sequence is in upper case, intron sequence is in lower case. (B) Shows the location of splice sites within the VEGF-X cDNA sequence. The location of mRNA splicing events is indicated by vertical lines. The cryptic splice donor/acceptor site at nt. 998/999 (diagonal lines) gives rise to the splice variant forms of VEGF-X. No splice site information is given for the region shown in italics.
- Figure 31: is a graphic representation of the effect of FL-VEGF-X on HuVEC proliferation: (24 hour serum starvation followed by one day treatment).
- Figure 32: is a graphic representation of the combined effect of truncated VEGF-X (CUB domain) and human recombinant VEGF₁₆₅ on HuVEC proliferation: (24 hour serum starvation followed by two day treatment).
- Figure 33: is a graphic representation of the combined effect of the CUB domain and human recombinant bFGF on HuVEC proliferation: (24 hour serum starvation followed by two day treatment).
- Figure 34: is a graphic representation of the

results of a LDH assay for testing cytotoxicity of the CUB domain or the CUB domain with rhVEGF₁₆₅.

- 5 Figure 35: is a graphic representation of the results obtained from a LDH assay for testing cytotoxicity of the CUB domain or CUB domain with rh-bFGF.
- 10 A BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990 J. Mol. Biol. 215, 403-410) search was performed in the proprietary LifeSeq™ human EST database (Incyte Pharmaceuticals, Inc., Palo Alto, CA, USA). BLAST produces alignments of both
- 15 nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologues. While it is useful for matches which do
- 20 not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).
- 25 Eighteen human EST clones (Figure 14) with high similarity to the previously identified VEGF proteins were identified and a further fifty EST clones (Figure 15) were identified using these sequences as query sequences, allowing us to deduce the putative
- 30 sequence for the new VEGF-X protein. The sequences obtained were compared to known sequences to determine regions of homology and to identify the sequence as a novel VEGF-type protein. Using the DNA sequence information in the databases we were able to
- 35 prepare suitable primers having the sequences of VEGF-X 1-10 illustrated in Figure 3 for use in subsequent RACE experiments to obtain the complete

- 29 -

DNA sequence for the VEGF-X gene.

Cloning

5 A profile was developed based on the VEGF-like domain, in existing VEGF sequences (VEGF-A, B, C and D). This was used to search the public databases and the Incyte LifeSeq™ database. No significant novel matching sequences were found in the public
10 databases. All of the matching sequences found in the LifeSeq™ database (~1000) were assembled to give a smaller number of sequences (~30), which included the known VEGFs and a potential novel VEGF (figures 1 and 2). This sequence was named VEGF-X.

15 Oligonucleotides were designed to amplify the VEGF-X sequence from cDNA (figure 3). The ESTs found in LifeSeq™ were from a range of tissues, with a slight predominance of sequences from ovary, testis,
20 placenta and lung (Figure 14 and 15). Accordingly the oligonucleotides were used to amplify cDNA derived from lung and placenta. First-round PCR products were found at ~200bp larger than the expected sizes, while 3 major species appeared after
25 a second round of PCR amplification, the smallest of which was of the expected size. These fragments were cloned and sequenced. The smallest fragment did indeed have the sequence originally identified from the LifeSeq database, while the others contained
30 insertions (figure 4).

As the first round of amplification suggested that the major species found in cDNA from ovary and placenta was not that originally identified in the
35 LifeSeq™ database, the focus of effort was switched to the presumed major species (it seemed likely that

- 30 -

clones 57, 25-27 and 2.1kb clones 1-3 in fig 4 represented the major mRNA species). Conceptual translation of the DNA sequences of these cloned PCR fragments indicated that the complete open reading frame was not present in the clones or in the sequence from LifeSeq™. While all clones contained the same sequence in the region of the translation termination codon, indicating that the end of the open reading frame had been identified, the 5' end of the open reading frame had not been cloned. 5' RACE experiments were therefore carried out in order to find the start of the reading frame. PCR primers designed for RACE experiments are shown in figure 5. RACE PCR products were sequenced directly. Sequence could be obtained from the 3' end of these RACE products but not from the 5' end; probably because the products were not cloned and were therefore heterogeneous at the 5' end. This new sequence was assembled with the existing cloned sequence to give the sequence shown in figure 6. Searching the LifeSeq™ database with this sequence identifies ESTs which extend the sequence a further 140bp in the 5' direction and a further 160bp in the 3' direction (figure 7). This longer contig was used to design oligonucleotide primers to amplify the entire coding sequence (these primer sequences are shown in figure 8). PCR was carried out using primers 5'-1 and vegfX10 (in order to clone a "full-length" cDNA), and with primers 5'-1 and vegfX6 (in order to clone the full coding region, see figure 3 for sequences of vegfX10 and vegfX6). A number of clones were obtained for the shorter fragment, of which clones 4 and 7 contain no PCR errors (sequence of clones 4 & 7 in figure 9). A single clone was obtained for the longer fragment (clone 9), but this sequence appears to contain 2 PCR errors.

The predicted polypeptide from these longer contigs is shown in figure 10. Amino acids 1-22 are predicted to encode a signal sequence (von Heijne, 5 1986, *Nucleic Acids Res.* 14, 4683-4690). Figure 11 shows an alignment of the protein sequence with VEGFs A-D. The region homologous to the other VEGFs is located towards the C-terminus of the protein. As the VEGF homology domain is expected to belong to the 10 TGF-beta superfamily of growth factors and to consist of a dimer containing both intra- and intermolecular disulphide bonds, initial alignments focussed on the cysteines. However, mapping of the sequence onto the known x-ray structure of the VEGF-A receptor-binding 15 domain (Muller et al (1997) *Proc. Natl. Acad. Sci USA* 94, 7192-7197) suggests that the alignment in figure 11 is plausible, as the extra 4 cysteine residues within the VEGF-homology region of VEGF-X (compared to this region of VEGF-A) correspond to residues 20 which are spatially close in VEGF-A, and may therefore be able to form disulphide bonds.

A search of the PFAM database of protein domains with the full-length polypeptide sequence from figure 10 25 identifies two domain consensus sequences within the polypeptide. The more C-terminal domain is a "VEGF" domain: (the known VEGFs all contain this domain and the structure of this region of VEGF-A is similar to that of PDGF). Additionally towards the N-terminus 30 of the polypeptide there is a CUB domain (amino acids ~40-150). The CUB domain is a 100-110 amino acid extracellular domain found in a number of developmentally-regulated proteins. When the full-length protein is used to search the protein 35 databases using the BLAST 2 algorithm, the scores for matches to CUB domain-containing proteins are more

- 32 -

significant than those to the other VEGFs. Interestingly, the most significant matches are to the CUB domains of Neuropilins, and Neuropilin-1 was recently identified as a receptor of one of the VEGF-A isoforms VEGF-A₁₆₅ (Soker et al. (1998) *Cell* 92, 735-745).

Assuming that the variant sequences isolated by PCR (i.e. the smaller PCR fragments) use the same 10 translation initiation site as the full-length sequence, they would result in production of the variant proteins shown in figure 12. It may be significant that both of these variant proteins retain the CUB domain and delete all or part of the 15 VEGF-like domain. The production of these variant sequences can be explained by the use of a cryptic splice donor/acceptor site within the VEGF-X sequence (figure 30B, between nt. 998/999): one variant arises by splicing out of the region between nt. 729-998, 20 the other by splicing out of the region between nt. 999-1187.

Expression

25 **Full-length expression constructs**

Mammalian cells

Clone 4 containing the full CDS of VEGF-X (see figure 9), was used to generate constructs for expression of 30 full-length protein. The sequence was amplified by PCR and cloned into the vector pCDNA6/V5-His so as to add a C-terminal V5 epitope tag and His₆ tag. The DNA and polypeptide sequence in this vector is shown in figure 19. Transient expression in COS cells followed by western blotting and detection via an 35 anti-V5 mAb demonstrates the secretion of a protein of ~50K into the medium in transfected cells only

(figure 22A). This construct can also be used to generate VEGF-X expressing stable CHO cell lines.

Baculovirus/Insect-cell expression system

5 For expression in the baculovirus/insect cell system the DNA encoding the predicted mature VEGF-X polypeptide sequence was fused to a sequence encoding a signal derived from melittin, a secreted insect protein. An N-terminal 6His tag was also added to
10 facilitate purification. The insert was then cloned into the baculovirus expression vector pFASTBAC. The DNA and polypeptide sequence of this construct is shown in figure 20. Infection of *Trichoplusia ni* Hi5 cells with this recombinant baculovirus results in
15 the secretion of a protein of approximately 45K into the medium (data not shown).

E.coli

20 The coding region of VEGF-X has been cloned in a variety of ways for expression as a secreted protein in *E.coli*. A particularly useful expression clone carries an N-terminal fusion to the *E.coli* maltose-binding protein (MBP- derived from the expression vector pMAL-p2, New England Biolabs) and a
25 C-terminal fusion to a 6His tag. The DNA and polypeptide sequence of this vector is shown in figure 21. Sequential purification of cell fractions on Ni-NTA resin and amylose resin allows the isolation of the expressed protein (see figure 22B).
30

Expression of fragments

VEGF

35 The VEGF domain of VEGF-X has been expressed in *E.coli*. Similar domains from VEGF-A (Christinger et al. (1996) *PROTEINS: Structure, Function and Genetics* 26, 353-357), and VEGF-D (Achen et al (1998) *Proc.*

- 34 -

Natl. Acad. Sci USA 95, 548-553) have been shown to be capable of binding to the respective receptors. Expression of these domains was carried out using the bacterium *E.coli*. Additionally, the full-length protein was expressed using the baculovirus/insect cell expression system. The oligonucleotide primers which have been obtained for these experiments are shown in figure 13. The construct directed expression in the bacterial cytoplasm, and as expected the protein was produced in insoluble form in inclusion bodies (the DNA and polypeptide sequence used for PDGF domain expression is shown in figure 24). Inclusion bodies were washed, solubilized with urea and the protein purified under denaturing conditions, before refolding by dialysis to remove the urea. Soluble protein was obtained, but shows little evidence of the disulphide bond linked dimers seen with material derived from animal cells (figure 25, compare with figure 22A & B). It is not clear therefore whether this protein is correctly folded.

CUB

The CUB domain has been expressed as a soluble secreted protein in *E.coli* (figure 26). The protein was purified by binding to Ni-NTA resin (figure 27) and assayed for activity on HUVECs in an in-vitro proliferation assay.

Properties of the VEGF-X protein

The transient mammalian cell expression system described above has been used to generate full-length VEGF-X protein, as shown by antibody detection following Western blotting (see figure 22A).

Disulphide bond linked dimers

The other members of the PDGF family of growth

- 35 -

factors, the PDGFs and VEGFs, all exist as dimers in which two monomers constituting the dimer are linked by interchain disulphide bonds. The x-ray structures of PDGF-BB (Oefner et al, 1992), and VEGF-A (Muller et al, 1997) are known and indicate that at least these two members of the family contain two interchain disulphide bonds. Practically this means that in SDS-PAGE analysis of these growth factors the presence of interchain disulphide bonds is shown by a large decrease in mobility in the absence of reducing agent (ie. the nonreduced dimer migrates more slowly through the gel than the reduced monomer). This effect was also expected for VEGF-X, and has been demonstrated for the material obtained from transient mammalian cell expression (figure 22A). In the case of the full length material produced in *E.coli* only some 10% of the total VEGF-X protein appears to be present as disulphide bond-linked dimers (figure 22B). However, these results provide evidence that the mammalian cell-derived protein is correctly folded, and that a portion of the *E.coli*-derived protein is too.

Glycosylation

There are 3 predicted potential N-linked glycosylation sites within the VEGF-X protein: at residues 25, 55 and 254 of the polypeptide sequence. The predicted molecular mass of the mature VEGF-X protein is 40kDa, but SDS-PAGE and western blotting (detection via an introduced C-terminal epitope tag- see figure 19) of the full-length protein expressed in COS cells gives a band slightly larger than the expected size (45-50kDa) as well as one at 25kDa (figure 22A). This smaller band is presumed to be a C-terminal proteolysis fragment derived from the full-length molecule (controls from uninfected cells do not show this band), probably corresponding to a

- 36 -

cleavage between the CUB and VEGF domains. EndoH treatment of the preparation gives a slight mobility change for the full-length protein (figure 23), but for the smaller VEGF domain fragment there is a clear
5 change, indicating that the predicted glycosylation site within the VEGF domain at residue 254 is indeed glycosylated.

Activity of proteins in cell-based assays
10 Protein samples were tested for activity in cell proliferation, cell migration and in-vitro angiogenesis assays. Active samples can also be tested in the in vivo matrigel mouse model of angiogenesis.
15

Full-length VEGF-X protein
Conditioned medium derived from COS cells transiently expressing VEGF-X (see figure 22A) displayed no detectable activity in any of the assays. However,
20 as VEGF-X protein could only be detected in this preparation by Western blotting, and not by Coomassie-staining of gels, it is clearly present at very low levels and this may be the reason for the observed lack of activity in the cell proliferation,
25 migration or in vitro angiogenesis tests.

VEGF domain
The VEGF domain protein described above has been tested in cell proliferation (on a range of cell types), cell migration and in vitro angiogenesis assays and has failed to show activity in any of these tests. As suggested above, this may be due to incorrect folding of this protein.
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35 CUB domain
The CUB domain protein at the highest dose tested

- 37 -

(1 μ g/ml) appears to inhibit proliferation of HUVECs in the absence of other stimulation (figure 28A & B). This effect is also seen following stimulation with the lowest VEGF-A₁₆₅ dose tested (1ng/ml- figure 28C).
5 The CUB domain of VEGF-X therefore appears to show antiproliferative activity on HUVECs, even in the presence of low VEGF-A₁₆₅ doses.

Tissue distribution of mRNA

10 VEGF-A mRNA expression has been shown to be upregulated in a wide variety of human tumors (lung, breast, ovarian, colon, stomach, liver, pancreas, kidney, bladder and prostate- Takahashi et al, 1995). Tumor VEGF-A expression has been shown to correlate
15 with tumor growth rate, microvascular density and tumor metastasis (Takahashi et al, 1995). It was thus of interest to examine the mRNA expression patterns of VEGF-X. Accordingly, Northern blot analysis of mRNA derived from different tissues has
20 been carried out. The results indicate that although the VEGF-X mRNA is expressed at low levels, it is present in a wide range of tissues. PCR amplification of cDNA from a range of tissue sources supports this idea (figure 29A). The major mRNA
25 species is approximately 3.1kb in size. There is no significant upregulation seen in tumour cell lines or in tumour tissues tested (figure 29B), with the possible exception of the cell lines GI-117 (lung carcinoma) and SaOS-2 (osteosarcoma). The results of
30 these initial tissue distribution studies do not, therefore, provide evidence for upregulation of VEGF-X in tumour growth, as is seen with VEGF-A.

Genomic structure of the VEGF-X gene

35 A genomic BAC clone covering the 3' part of the VEGF-X locus was isolated by hybridisation screening

- 38 -

of nylon filters containing a human BAC library. Direct sequencing of this clone using oligonucleotide primers based on the VEGF-X cDNA sequence allowed the determination of several intron/exon boundaries
5 (figure 30). Interestingly, the position of the mRNA splice site within the PDGF domain (nt 1187/1188 in figure 30B) is conserved with respect to those in the VEGF-A and VEGF-D genes (Tischer et al, 1991; Rocchigiani et al, 1998).

10

Materials & Methods

PCR, Cloning, DNA sequence determination and BAC screening.
15 All primers were purchased from Eurogentec, Seraing, Belgium. Insert-specific sequencing primers (15- and 16-mers) were designed by visual inspection of the DNA sequences. DNA was prepared on Qiagen-tip-20 columns or on Qiaquick spin columns (Qiagen GmbH, Düsseldorf, Germany) and recovered from the spin columns in 30µl Tris/EDTA-buffer (10mM TrisHCl pH 7.5, 1 mM EDTA (sodium salt)). Sequencing reactions were performed using BigDye™ Terminator Cycle Sequencing Ready Reaction kits (Perkin Elmer, ABI Division, Foster City, CA, USA) and were run on an Applied Biosystems 377 DNA sequencer (Perkin Elmer, ABI Division, Foster City, CA, USA).
20 Polymerase chain reactions were carried out according to standard procedures (Ausubel et al, 1997). The PCR fragments were cloned into vectors pCR2.1 (Perkin Elmer, ABI Division, Foster City, CA, USA).
25 Polymerase chain reactions were carried out according to standard procedures (Ausubel et al, 1997). The PCR fragments were cloned into vectors pCR2.1 (Invitrogen, Carlsbad, CA, USA) or pCR-TOPO (Invitrogen, NL) according to the manufacturer's instructions. One of those vectors, plasmid VEGFX/pCR2.1 1TOPO FL
30 was deposited on 1 March 1999 under Accession No. LMBP 3925. After sequence determination, the inserts were cloned into the desired expression vectors (see
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- 39 -

figures 19, 20, 21, 24 & 26).

A human genomic BAC library (Genome Systems, Inc., St Louis, MI, USA) was screened by hybridisation to
5 oligonucleotides derived from the VEGF-X cDNA sequence, according to the manufacturer's instructions. BAC DNA was prepared using a Qiagen plasmid midi kit (Qiagen GmbH, Düsseldorf, Germany) according to the manufacturer's instructions with
10 some modifications (after clearing of the lysate from chromosomal DNA, supernatants from individual preparations were pooled on a single column (tip 100), and after the 70 % EtOH wash, the pellet was resuspended overnight at 4°C in 100 µl TE). 20-mer
15 sequencing primers were designed based on the known cDNA sequence, and sequencing carried out as above.

5' RACE

20 In order to extend the cDNA clone in a 5' direction RACE reactions were carried out. Since it was known that the mRNA is present in placenta and skeletal muscle, Marathon-Ready™ placenta and skeletal muscle cDNAs were purchased from Clontech (Palo Alto CA.
25 USA) and used according to the manufacturer's instructions. DNA fragments were excised from agarose gels, purified using QiaQuick PCR purification columns (Qiagen GmbH, Düsseldorf, Germany) and sequenced directly.

30 VEGF-X protein expression and purification DNA fragments encoding the desired protein sequences were amplified by PCR and cloned into appropriate expression vector systems.

35 For mammalian cell expression, the full coding

- 40 -

sequence was cloned into the vector pcDNA6/V5-his (Invitrogen Leek, NL, see figure 19 for construct sequence), so as to add a C-terminal peptide tag to assist in detection and purification.

5

For insect cell expression the sequence of the predicted mature polypeptide was initially amplified to add an N-terminal 6His peptide and then cloned into the pMelBacB vector (Invitrogen, Leek, NL) to add an insect cell signal sequence. The entire insert was then PCR-cloned into the vector pFASTBAC-1 (LifeTechnologies, Gaithersburg, MA, USA) for construction of a baculovirus according to the manufacturer's instructions.

10

For *E.coli* expression, the coding region was PCR amplified to add a C-terminal 6His tag and then cloned into the vector pMAL-p2 (New England Biolabs, Beverly, MA, USA). The coding sequence of this construct is shown in figure 21). The protein was purified first on Ni-NTA resin (Qiagen GmbH, Düsseldorf, Germany) and then on amylose resin (New England Biolabs, Beverly, MA, USA), according to the manufacturers' instructions.

15

DNA sequences encoding the CUB and VEGF domain fragments of VEGF-X were PCR amplified and cloned into pET22b and pET21a (Novagen, Madison, WI, USA) respectively. The CUB domain protein was prepared either from the periplasm or medium of induced cultures by standard methods (Ausubel et al, 1997). The protein was initially purified by precipitation with 20% ammonium sulphate. After overnight dialysis vs 20mM Tris Hcl pH7.5, 100mM NaCl to remove ammonium sulphate, the protein was further purified on Ni-NTA resin as described above. The VEGF domain protein was expressed in insoluble form, and preparation of

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inclusion bodies was carried out using standard procedures (Ausubel et al 1997). Inclusion bodies were dissolved in 6M guanidine hydrochloride, 20mM Tris HCl pH8.0, 200mM NaCl, 1mM 2-mercaptoethanol, and purified on Ni-NTA resin (Qiagen GmbH, Düsseldorf, Germany) according to the manufacturer's instructions. The protein was refolded by dialysis against several changes of buffer containing decreasing concentrations of denaturant.

10

Analysis of protein glycosylation was carried out using EndoH (Roche Molecular Biochemicals, Brussels, BE) according to the manufacturer's instructions.

15

Cell Proliferation Assay

Human umbilical vein endothelial cells (HUVECs) (Clonetics, San Diego, CA.) were trypsinized with 0.05% trypsin/0.53mM EDTA (Gibco, Gaithersburg, MD.), resuspended in the EGM-2 (Clonetics, San Diego, CA.), counted, and distributed in a 96-well tissue culture plate at 5,000 cells/well. Following cell attachment and monolayer formation (16 hours), cells were stimulated with various concentrations of truncated VEGF-X (CUB domain or VEGF domain) or dilutions of

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culture supernatants of the full-length VEGF-X (COS 7 or HEK293) in DMEM (Gibco, Gaithersburg, MD.) containing 0.5% to 2% FBS (HyClone, Logan, UT) as indicated. For human fetal dermal fibroblasts

25

(American Type Culture Collection, Rockville, MD.),

30

the growth medium was replaced by DMEM containing 0.1% BSA (Sigma, St. Louise, MO.) with or without various concentrations of truncated VEGF-X proteins.

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For HCASMC (Clonetics, San Diego, CA.), the medium was replaced by DMEM containing 0.5% FBS. The cells were treated for a further 24 hr-72 hr. For the measurement of proliferation, the culture media were replaced with 100 µl of DMEM containing 5% FBS and 3

- 42 -

μ Ci/ml of [³H]-thymidine (Amersham, Arlington Heights, IL.). Following pulse labeling, cells were fixed with methanol/acetic acid (3:1, vol/vol) for 1 hour at room temperature. The cells were washed twice with 250 μ l/well of 80% methanol. The cells were solubilized in 0.05% trypsin (100 μ l/well) for 30 minutes then in 0.5% SDS (100 μ l/well) for another 30 minutes. Aliquots of cell lysates (180 μ l) were combined with 2 ml of scintillation cocktail (Fisher, Springfiled, NJ) and the radioactivity of cell lysates was measured using a liquid scintillation counter (Wallac 1409). In each case, samples were performed in quadruplicate.

15 **Chemotaxis Assay**
The chemotactic response of HUVECs was assayed using a 48-well modified Boyden chamber (NeuroProbe, Cabin John, MD.) and collagen-coated (0.1mg/ml type I collagen, Collaborative Biomedical, Bedford, MA.) polycarbonate membrane filters with a pore diameter of 8 μ m (NeuroProbe, Cabin John, MD.). Cell suspensions (15,000/well) were loaded to the upper part of the chemotaxis chamber and stimulated for 4 hours with rhVEGF₁₆₅ (0.1-10 ng/ml) (Calbiochem, San Diego, CA.) or various concentrations of truncated VEGF-X (PDGF domain). Cells remaining on the top of the membrane were removed. Migration was assessed by counting the number of cells that migrated to the lower side of the filter membrane. The membrane was fixed with 10% formaldehyde for 15 min, followed by staining with Gill's hematoxylin III (Poly Scientific, Bay Shore, NY.). The assay was performed in triplicates and six independent high power fields per well were counted using a light microscope at 250 magnification. The results were expressed as the fold of unstimulated cells (EGM containing 0.1% BSA).

- 43 -

In Vitro Angiogenesis Assay

In vitro angiogenesis in fibrin gels was quantitated using spheroids of human umbilical vein endothelial cells (Korff et al., 1998). To generate endothelial cell spheroids of defined size and cell number, a specific number of cells (~ 800 cells per spheroid) was suspended in EGM-2 culture medium containing 20% methylcellulose (Sigma, St. Louis, MO.), seeded into nonadherent round-bottom 96-well plates. All suspended cells in one well contributed to the formation of a single endothelial cell spheroid within 24 hours. A fibrin gel stock solution was prepared freshly prior to use by mixing 3mg/ml fibrinogen (Calbiochem, San Diego, CA.) in Medium 199 (Gibco, Gaithersburg, MD.). Assays were performed in 24-well culture plates. The 1ml fibrinogen stock was mixed with 50 HUVEC spheroids and the corresponding test substance including rh-VEGF₁₆₅ or various concentration of VEGF-X. The spheroid-containing fibrinogen was rapidly transferred into 24-well plates. Fifteen microliters of thrombin (100 NIH U/ml stock, Sigma, St. Louis, MO.) was added to the gel for the fibrin gel formation. The gel formation usually occurred within 30 seconds. After gel formation, 1ml/well of Medium 199 supplemented with 20% FBS, 1mg/ml ϵ -aminocaproic acid (Calbiochem, San Diego, CA.) and antibiotics were added. The gel was incubated at 37°C (5%CO₂, 95% air, 100% humidity). After 3 days, *in vitro* angiogenesis was quantitated by measuring the length of the three longest capillary sprouts that had grown out of each spheroid (100X magnification), analyzing at least 10 spheroids per experimental group and experiment.

35

Matrigel Mouse Assay

- 44 -

The matrigel mouse assay is carried out as described by Passanti et al (1992).

5 **Analysis of VEGF-X gene expression by RT-PCR analysis.**

Oligonucleotide primers VEGF-E2 and VEGF-X14 (figure 16; figure 5) were used for the specific PCR amplification of a 350 bp fragment from VEGF-X. PCR amplifications were performed on human multiple 10 tissue cDNA (MTCTM) panels (Clontech human MTC panels I and II and human Tumor MTC panel) normalised to the mRNA expression levels of six different housekeeping genes. In addition, cDNA was made from different tumor cell cultures (Caco-2 colorectal 15 adenocarcinoma; T-84 colorectal carcinoma; MCF-7 breast adenocarcinoma; T-47D breast ductal gland carcinoma; HT1080 bone fibrosarcoma; SaOS-2 osteosarcoma; SK-N-MC neuroblastoma; HepG2 hepatoblastoma; JURKAT T-cell leukemia and THP-1 20 myelomonocytic leukemia). For the preparation of tumor cell cDNA, cells were homogenised and total RNA prepared using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. 1 µg of total RNA was reverse 25 transcribed using oligo(dT)15 as a primer and 50 U of ExpandTM Reverse Transcriptase (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions. PCR reactions with VEGF-X-specific or glyceraldehyde-3-phosphate dehydrogenase 30 (G3PDH)-specific primers were then performed on 1 µl of this cDNA. For all cDNAs, PCR reactions with VEGF-X specific primers were performed in a total volume of 50 µl, containing 5 µl (\pm 1 ng) of cDNA, 1x Advantage KlenTaq PCR reaction buffer, 0.2 mM dNTP, 35 250 nM of primers VEGF-E2 and VEGF-X14 and 1 µl of Advantage KlenTaq polymerase mix. Samples were heated

- 45 -

to 95°C for 30 s and cycling was done for 30 s at 95°C and 30 s at 68°C for 25, 30 or 35 cycles. Control reactions using specific primers that amplify a 1 kb fragment of the housekeeping gene G3PDH were
5 also performed according to the manufacturer's instructions.

Northern blot analysis of VEGF-X.

10 Northern blots containing 2 µg of poly(A)-rich RNA derived from different human tissues (Clontech Laboratories; MTN™ blot, MTN™ blot II and Cancer Cell Line MTN™ blot) were hybridized according to the manufacturers instructions with a α-[³²P]-dCTP random-priming labelled (Multiprime labelling kit, Roche Diagnostics) 293 bp specific VEGF-X fragment (PinAI-StuI fragment including 92 bp of the 3' end coding region and 201 bp of the 3' untranslated region of VEGF-X). The blots were hybridized overnight at 68°C and final washes at high stringency
15 were at 68°C in 0.1x SSC/0.1 % SDS. The membranes were autoradiographed for 1 to 3 days with intensifying screens.

Full length VEGF-X

20 The effect of full length VEGF-X on proliferation of HuVEC cells was determined by the ³H-Thymidine incorporation assay. HuVEC cells were serum starved for 24 hours prior to treatment with the full length VEGF-X at the concentration range from 100 pg/ml-10 µg/ml. There was no effect of VEGF-X at 100 pg/ml-10 ng/ml on endothelial cell proliferation. At the higher concentrations of FL-VEGF-X (100 ng/ml and 1 µg/ml) there was a marked inhibition of endothelial cell proliferation. This is probably due to the very
25 high endotoxin level in the samples. The VEGF-X sample was purified in order to decrease the

- 46 -

endotoxin level and is currently tested in the cell proliferation assay.

The Summary from Testing the CUB Domain

5 The effect of CUB domain on inhibition of HuVEC
proliferation either serum- (2%), rh-VEGF or bFGF-
stimulated, was assessed by the ^3H -Thymidine
incorporation assay. Cells were serum starved
followed by the treatment with the CUB domain and
10 various growth factors. Results showed that the CUB
domain inhibited endothelial cell proliferation,
either serum- (2%), rh-VEGF or bFGF-stimulated in a
dose dependent manner with maximal inhibition at 10
15 $\mu\text{g}/\text{ml}$. There was approximately a 2-fold inhibition
of proliferation (at 10 $\mu\text{g}/\text{ml}$) of cells stimulated
with VEGF and bFGF and nearly a 5-fold inhibition of
cells stimulated with serum (2%). Results with the
LDH assay showed that there was no cytotoxicity
20 associated with the inhibition of cell proliferation
by the CUB domain.

Therefore, the N-terminus of the polypeptide from Figure 10 has been shown to possess a CUB domain. When database searches are carried out using the
25 full-length coding sequence the best matches (i.e. for a BLAST search, those with the lowest probability score) are found with the CUB domain rather than with the VEGF-like domain. The best match from searching release 37 of the SWISSPROT database (Feb 1999) is to the CUB domain of a neuropilin from *Xenopus laevis*, and the matches to the CUB domains of human
30 neuropilins 1 and 2 are also more significant than matches to the VEGFs.

35 This similarity is provocative, given the identification of neuropilin-1 and -2 as cellular receptors for the VEGF-A 165 (Stoker et al. 1998,

reviewed in Neufeld et al. 1999). It is plausible therefore that VEGF-X could exert dual regulatory effects: via interaction with the tyrosine kinase VEGF-receptors mediated by the VEGF-like domain, as well as via interaction with VEGF isoforms or with the neuropilin receptors, mediated by the CUB domain.

To the best of our understanding the latter would be entirely novel, and searches on the most recent release of the Incyte database do not reveal any other proteins containing both CUB and VEGF-like domains. This arrangement of domains suggests possible positive or negative models of regulation:

Positive- the VEGF-like domain is able to interact productively with the tyrosine kinase VEGF receptors giving activation, and the CUB domain is able to interact productively with the neuropilin receptor giving activation.

Negative- the VEGF-like domain does not interact productively with the tyrosine kinase VEGF receptors, either preventing receptor dimerisation or blocking the VEGF binding sites. Further, the CUB domain does not interact productively with the neuropilin receptors, either preventing receptor activation or blocking the VEGF binding sites, or indeed by binding to VEGF isoforms and preventing their interaction with receptors.

TABLE 1

	<u>ORIGINAL RESIDUE</u>	<u>EXEMPLARY SUBSTITUTIONS</u>
	ALA	SER, THR
5	ARG	LYS
	ASN	HIS, SER
	ASP	GLU, ASN
	CYS	SER
	GLN	ASN, HIS
10	GLU	ASP, GLU
	GLY	ALA, SER
	HIS	ASN, GLN
	ILE	LEU, VAL, THR
	LEU	ILE, VAL
15	LYS	ARG, GLN, GLU, THR
	MET	LEU, ILE, VAL
	PHE	LEU, TYR
	SER	THR, ALA, ASN
	THR	SER, ALA
20	TRP	ARG, SER
	TYR	PHE
	VAL	ILE, LEU ALA
	PRO	ALA

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SEQUENCE LISTING

- Sequence ID No 1 corresponds to the amino acid sequence from position 23 to 345 of the amino acid sequence illustrated in Figure 10.
- 5
- Sequence ID No 2 is the amino acid sequence illustrated in Figure 10.
- 10 Sequence ID No 3 corresponds to the sequence from position 257 to 1291 of the nucleotide sequence illustrated in Figure 9.
- 15 Sequence ID No 4 corresponds to the polynucleotide sequence of VEGFX1 illustrated in Figure 3.
- 20 Sequence ID No 5 corresponds to the polynucleotide sequence of VEGFX2 illustrated in Figure 3.
- 25 Sequence ID No 6 corresponds to the polynucleotide sequence of VEGFX3 illustrated in Figure 3.
- 30 Sequence ID No 7 corresponds to the polynucleotide sequence of VEGFX4 illustrated in Figure 3.
- 35 Sequence ID No 8 corresponds to the polynucleotide sequence of VEGFX5 illustrated in Figure 3.
- Sequence ID No 9 corresponds to the polynucleotide sequence of VEGFX6 illustrated in

- 53 -

Figure 3.

- Sequence ID No 10 corresponds to the polynucleotide sequence of VEGFX7 illustrated in Figure 3.
- 5
- Sequence ID No 11 corresponds to the polynucleotide sequence of VEGFX8 illustrated in Figure 3.
- 10 Sequence ID No 12 corresponds to the polynucleotide sequence of VEGFX9 illustrated in Figure 3.
- 15 Sequence ID No 13 corresponds to the polynucleotide sequence of VEGFX10 illustrated in Figure 3.
- Sequence ID No 14 corresponds to the polynucleotide sequence of VEGFX11 illustrated in Figure 4.
- 20
- Sequence ID No 15 corresponds to the polynucleotide sequence of VEGFX12 illustrated in Figure 4.
- 25
- Sequence ID No 16 corresponds to the polynucleotide sequence of VEGFX13 illustrated in Figure 4.
- 30 Sequence ID No 17 corresponds to the polynucleotide sequence of VEGFX14 illustrated in Figure 4.
- 35 Sequence ID No 18 corresponds to the polynucleotide sequence 5'-1 in Figure 8.

- Sequence ID No 19 corresponds to the polynucleotide sequence 5'-2 in Figure 8.
- 5 Sequence ID No 20 corresponds to the polynucleotide sequence of VEGFX6 illustrated in Figure 13.
- 10 Sequence ID No 21 corresponds to the polynucleotide sequence of VEGFX7 illustrated in Figure 13.
- 15 Sequence ID No 22 corresponds to the polynucleotide sequence of VEGFX8 illustrated in Figure 13.
- 20 Sequence ID No 23 corresponds to the polynucleotide sequence of VEGFX9 illustrated in Figure 13.
- 25 Sequence ID No 24 corresponds to the polynucleotide sequence of VEGBAC1 illustrated in Figure 13.
- 30 Sequence ID No 25 corresponds to the polynucleotide sequence of VEGBAC2 illustrated in Figure 13.
- 35 Sequence ID No 26 corresponds to a polypeptide having the amino acid sequence from amino acid position 40 to 150 of the sequence of Figure 10.
- 35 Sequence ID No 27 corresponds to a polypeptide having the amino acid sequence illustrated in Figure 26.
- Sequence ID No 28 corresponds to the sequence from

position 5 to 508 of the nucleotide sequence illustrated in Figure 26.

- 5 Sequence ID No 29 corresponds to the nucleotide sequence from position 5 to 508 of the nucleotide sequence illustrated in Figure 26.
- 10 Sequence ID No 30 corresponds to the sequence from position 214 to 345 of the nucleotide sequence illustrated in Figure 10.

CLAIMS

1. A nucleic acid molecule encoding a VEGF-X protein or a functional equivalent, derivative or bioprecursor thereof, said protein comprising any of the sequences from position 23 to 345 of the amino acid sequence illustrated in Figure 10, or the complete sequence as illustrated in Figure 10.
- 10 2. A nucleic acid molecule according to claim 1 wherein said nucleic acid is a DNA molecule.
- 15 3. A nucleic acid molecule according to claim 1 or 2 wherein said nucleic acid is a cDNA molecule.
- 15 4. A nucleic acid molecule according to claim 3 comprising the nucleotide sequence from position 257 to 1291 of the nucleotide sequence illustrated in Figure 9, or sequences that hybridise thereto under high stringency conditions or the complement thereto.
- 20 5. An antisense molecule capable of hybridising to a molecule according to any of claims 1 to 4 under high stringency conditions.
- 25 6. A nucleic acid molecule according to any of claims 1 to 4 which is of mammalian origin.
- 30 7. A nucleic acid molecule according to claim 6 which is of human origin.
- 35 8. An isolated VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, having an amino acid sequence from position 23 to 345 of the amino acid sequence illustrated in Figure 10 or the complete amino acid sequence of Figure 10.

9. A VEGF-X protein, or a functional equivalent, derivative or bioprecursor thereof, encoded by a nucleic acid molecule as defined in any of claims 1 to 4.
- 5
10. A protein according to claim 9, which comprises the amino acid sequence illustrated in Figure 10.
11. An expression vector comprising a nucleic acid molecule according to any of claims 1 to 4.
- 10
12. An expression vector according to claim 11 further comprising a nucleotide sequence encoding a reporter molecule.
- 15
13. An expression vector comprising an antisense molecule according to claim 5.
14. A nucleic acid molecule according to any of claims 1 to 4 or an antisense molecule according to claim 5 for use as a medicament.
- 20
15. A host cell transformed or transfected with an expression vector according to claim 11 or 12.
- 25
16. A host cell transformed or transfected with an expression vector according to claim 13.
17. A transgenic cell, tissue or organism comprising a transgene capable of expressing a VEGF-X protein according to claim 8 or 9.
- 30
18. A transgenic cell, tissue or organism according to claim 17, wherein said transgene is included in an expression vector.
- 35
19. A VEGF-X protein or a functional equivalent,

- 58 -

derivative or bioprecursor thereof, expressed by a cell according to claim 15.

20. A VEGF-X protein, or a functional equivalent,
5 derivative or bioprecursor thereof, expressed by a transgenic cell, tissue or organism according to claim 17.

21. A process for producing a VEGF-X protein
10 according to any of claims 8 to 10, said process comprising transforming a host cell or organism with an expression vector according to claim 11, and recovering the expressed protein from said host cell or organism.

15 22. An antibody capable of binding to a protein according to any of claims 8 to 10, or an epitope thereof.

20 23. An antibody according to claim 22 for use as a medicament.

25 24. A pharmaceutical composition comprising an antibody according to claim 22 together with a pharmaceutically acceptable carrier diluent or excipient thereof.

30 25. A method of identifying VEGF-X protein in a sample which method comprises contacting said sample with an antibody according to claim 22 and monitoring for binding of any protein to said antibody.

35 26. A kit for identifying the presence of VEGF-X protein in a sample which comprises an antibody according to claim 22 and means for contacting said antibody with said sample.

- 59 -

27. A method of identifying compounds which modulate angiogenesis which method comprises providing a host cell or organism according to claim 15 or a transgenic cell, tissue or organism according to
5 claim 17, contacting a test compound with said cell, tissue or organism and monitoring for an effect of said compound on said VEGF compared to a host cell or organism according to claim 15 or a transgenic cell tissue or organism according to claim 17 which has
10 not been contacted with said compound.

28. A compound identifiable according to the method of claim 27.

15 29. A compound according to claim 28 for use as a medicament.

20 30. A nucleic acid sequence comprising the nucleotide sequences illustrated in any of Figures 3,
5, 8 or 13.

31. A method for producing a polypeptide, said method comprising the steps of:

25 a) culturing the host cell of claim 15 under conditions suitable for expression of the polypeptide; and
b) recovering the polypeptide from the host cell culture.

30 32. A method of inhibiting angiogenic activity and inappropriate vascularisation including formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ
35 and tissue repair in a subject said method comprising administering to said subject an amount of an antisense molecule according to claim 5 in sufficient

- 60 -

concentration to reduce or prevent said angiogenic activity.

33. A method of inhibiting angiogenic activity or
5 inappropriate vascularisation including any of
formation and proliferation of new blood vessels,
growth and development of tissues, tissue
regeneration and organ and tissue repair in a subject
said method comprising administering to said subject
10 an amount of an antibody according to claim 22 in
sufficient concentration to reduce or prevent said
angiogenic activity or inappropriate vascularisation.

34. A method of inhibiting angiogenic activity or
15 inappropriate vascularisation including any of
formation and proliferation of new blood vessels,
growth and development of tissues, tissue
regeneration and organ and tissue repair in a
subject, said method comprising implanting in said
20 subject cells that express an antibody according to
claim 22.

35. A method of treating or preventing any of
cancer, rheumatoid arthritis, psoriasis and diabetic
25 retinopathy, said method comprising administering to
said subject an amount of an antisense molecule
according to claim 5 in sufficient concentration to
treat or prevent said disorders.

30 36. A method of treating or preventing any of
cancer, rheumatoid arthritis; psoriasis and diabetic
retinopathy, said method comprising administering to
said subject an amount of an antibody according to
claim 22 in sufficient concentration to reduce or
35 prevent said disorders.

37. A method of promoting angiogenic activity or

- vascularisation to promote wound healing, skin graft growth, tissue repair, proliferation of new blood vessels, tissue regeneration and organ repair which method comprises applying or delivering to a site of interest a therapeutically effective amount of any of a group selected from a protein according to claim 8 and a nucleic acid molecule encoding a VEGF-X protein or a functional equivalent, derivative or bioprecursor thereof comprising an amino acid sequence illustrated in Figure 10, an expression vector comprising said nucleic acid molecule and a pharmaceutical composition comprising any of said nucleic acid molecule and said protein.
- 5 38. A method of treating wounds selected from the group consisting of dermal ulcers, pressure sores, venous sores, diabetic ulcers and burns by applying to said wound a therapeutically effective amount of any of a VEGF-X protein according to claim 8, a pharmaceutical composition comprising said protein and a pharmaceutically acceptable carrier, diluent or excipient therefor.
- 10 39. A nucleic acid molecule encoding a polypeptide having a CUB domain said polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence of Figure 10.
- 15 40. A nucleic acid molecule encoding a polypeptide having a CUB domain, said polypeptide comprising the amino acid sequence of Figure 26.
- 20 41. A nucleic acid molecule according to claim 39 or 40, comprising the nucleotide sequence from position 5 to 508 of the sequence illustrated in Figure 26.
- 25 42. A nucleic acid molecule according to any of

- 62 -

claims 39 to 41 comprising the nucleotide sequence illustrated in Figure 26.

43. A nucleic acid molecule encoding a VEGF like
5 domain comprising the sequence from position 214-345
of the sequence of Figure 10 or the sequence from
position 15 to 461 illustrated in Figure 24.

44. An expression vector comprising a nucleic acid
10 molecule according to any of claims 39 to 42.

45. An expression vector comprising a nucleic acid
molecule according to claim 43.

15 46. A host cell transformed or transfected with an
expression vector according to claim 44.

47. A host cell transformed or transfected with an
expression vector according to claim 45.

20 48. A protein expressed by the cell according to
claim 46.

49. A protein expressed by the cell according to
25 claim 47.

50. A method of identifying compounds that inhibit
or enhance angiogenic activity, said method
comprising contacting a cell expressing a VEGF
30 receptor and/or a neuropilin 1 or 2 type receptor
with said compound in the presence of a VEGF-X
protein according to claim 8 and monitoring for the
effect of said compound or said cell when compared to
a cell which has not been contacted with said
35 compound.

51. A compound identifiable according to the method

of claim 50 as an inhibitor or enhancer of angiogenic activity.

5 52. A method of inhibiting angiogenic activity or inappropriate vascularisation, said method comprising contacting a cell expressing a VEGF receptor and a neuropilin type receptor with a protein selected from any of a protein according to any of claims 8 to 10 and a protein according to claim 48 or a protein according to claim 49.

10 53. Use of a nucleotide sequence illustrated in any of Figures 14 and 15 in identifying a VEGF-X protein according to claim 8.

15 54. A nucleic acid molecule encoding a polypeptide comprising a CUB domain having the sequence from position 40 to 150 of the sequence of Figure 10 or from position 5 to 508 of the sequence of Figure 26 and a sequence encoding a VEGF domain.

20 55. A nucleic acid molecule according to claim 54 wherein said sequence encoding said VEGF domain is selected from the sequences encoding any of VEGF A to D or isoforms or variants thereof.

25 56. A nucleic acid molecule encoding a polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 for use as a medicament.

30 57. Use of a nucleic acid molecule encoding a polypeptide having the amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 in the manufacture of a medicament for treatment of disease conditions associated with inappropriate angiogenesis such as tumour or cancer

growth, retinopathy, osteoarthritis or psoriasis.

5 58. A polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence illustrated in figure 10 for use as a medicament.

10 59. A polypeptide comprising the amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 in the manufacture of a medicament for the treatment of disease conditions associated with inappropriate angiogenesis such as tumour growth, retinopathy, osteoarthritis or psoriasis.

15 60. Use of a CUB domain comprising the amino acid sequence from position 40 to 150 of the sequence of Figure 10, or the amino acid sequence of Figure 26, to identify compounds which inhibit angiogenic activity in a method according to claim 50.

20 61. A method of inhibiting angiogenic activity and inappropriate vascularisation including formation and proliferation of new blood vessels, growth and development of tissues, tissue regeneration and organ and tissue repair in a subject said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 or a nucleic acid molecule according to any of claims 39 to 42 in sufficient concentration to reduce or prevent said angiogenic activity.

25 62. A method of treating or preventing any of cancer, rheumatoid arthritis, psoriasis and diabetic retinopathy, said method comprising administering to said subject an amount of a polypeptide having an amino acid sequence from position 40 to 150 of the sequence illustrated in Figure 10 or a nucleic acid

- 65 -

molecule according to any of claims 39 to 42 in sufficient concentration to treat or prevent said disorders.

5 63. An antisense molecule capable of hybridising to a molecule according to any of claims 39 to 42 under high stringency conditions.

10 64. An antisense molecule capable of hybridising to a molecule according to claim 43 under high stringency conditions.

15 65. A transgenic cell, tissue or organism comprising a transgene capable of expressing a protein according to claim 48.

66. A transgenic cell, tissue or organism comprising a transgene capable of expressing a protein according to claim 49.

20 67. A transgenic, cell tissue or organism according to claim 65 or 66, wherein said transgene is included in an expression vector according to claim 41 or 42.

25 68. An antibody capable of binding to a protein according to claim 48 or an epitope thereof.

30 69. An antibody capable of binding to a protein according to claim 49 or an epitope thereof.

35 70. A pharmaceutical composition comprising an antibody according to claim 68 or 69 together with a pharmaceutically acceptable carrier diluent or excipient therefor.

71. A pharmaceutical composition comprising a

- 66 -

compound according to claim 48 together with a pharmaceutically acceptable carrier, diluent or excipient therefor.

- 5 72. A nucleic acid molecule encoding a variant of a VEGF-X protein having any of the sequences of nucleotides illustrated in Figure 12.

FIG. 1

1 AAAATGTATG GATACAACCTT ACGTTTGATG AAAGATTTGG GCTTGAAGAC CCAGAAGATG
TTTACATAC CTATGTTGAA TGCAAACATAC TTTCTAAACC CGAACCTCTG GGTCTTCTAC

61 ACATATGCAA GTATGATTG AGGAACCCAG TGATGGAAC ATATTAGGGC
TGTATACGTT CATACTAAAA CATCTCAAC TCCTTGGTC ACTACCTTGA TATAATCCCG

121 GCTGGTGTGG TTCTGGTACT GTACCAGGAA AACAGATTTC TAAAGGAAAT CAAATTAGGA
CGACCACACC AAGACCATGA CATGGCCTT TTGTCTAAAG ATTTCCTTA GTTTAACCT

+1 MetAsn IlePheLeu LeuAsnLeuLeu ThrGluGlu ValArgLeu
]-----

181 TAAGATTGT ATCTGATGAA TATTTCCCTT CTGAACCTTC TAACAGAGGA GGTAAGATTA
ATTCTAAACA TAGACTACTT ATAAAAGGAA GACTTGGAAAG ATTGTCTCCT CCATTCTAAT

+1 TyrSerCysThr ProArgAsn PheSerVal SerIleArgGlu GluLeuLys ArgThrAsp

241 TACAGCTGCA CACCTCGTAA CTTCTCAGTG TCCATAAGGG AAGAACTAAA GAGAACCGAT
ATGTCGACGT GTGGAGCATT GAAGAGTCAC AGGTATTCCC TTCTTGATTT CTCTGGCTA

+1 ThrIlePheTrp ProGlyCys LeuLeuVal LysArgCysGly GlyAsnCys AlaCysCys

301 ACCATTTCT GCCCAGGTTG TCTCCTGGTT AAACGCTGTG GTGGGAAC TGCGCTGTGT
TGGTAAAAGA CCGGTCCAAC AGAGGACCAA TTTGCGACAC CACCCTTGAC ACGGACAAACA

+1 LeuHisAsnCys AsnGluCys GlnCysVal ProSerLysVal ThrLysLys TyrHisGlu

361 CTCCACAATT GCAATGAATG TCAATGTGTC CCAAGCAAAG TTACTAAAAA ATACCACGAG
GAGGTGTTAA CGTTACTTAC AGTTACACAG GGTCGTTTC AATGATTTTT TATGGTGCTC

+1 ValLeuGlnLeu ArgProLys ThrGlyVal ArgGlyLeuHis LysSerLeu ThrAspVal

421 GTCCTTCAGT TGAGACCAAA GACCGGTGTC AGGGGATTGC ACAAAATCACT CACCGACGTG
CAGGAAGTCA ACTCTGGTTT CTGGCCACAG TCCCCTAACG TGTTAGTGA GTGGCTGCAC

+1 AlaLeuGluHis HisGluGlu CysAspCys ValCysArgGly SerThrGly Gly
----->

481 GCCCTGGAGC ACCATGAGGA GTGTGACTGT GTGTGCAGAG GGAGGCACAGG AGGATAGCCG
CGGGACCTCG TGGTACTCCTCACACTGACA CACACGTCTC CCTCGTGTCC TCCTATCGGC

541 CATCACCAAC AGCAGCTTT GCCCAGAGCT GTGCAGTGCA GTGGCTGATT CTATTAGAGA
GTAGTGGTGG TCGTCGAGAA CGGGTCTCGA CACGTACGT CACCGACTAA GATAATCTCT

601 ACGTATGCGT TATCTCCATC CTTAATCTCA GTTGTGTTGCT TCAAGGACCT TTCATCTTCA
TGCATACGCA ATAGAGGTAG GAATTAGAGT CAACAAACGA AGTTCCTGGA AAGTAGAAGT

661 GGATTACAG TGCATTCTGA AAGAGGAGAC ATCAAACAGA ATTAGGAGTT GTGCAACAGC
CCTAAATGTC ACGTAAGACT TTCTCCTCTG TAGTTGTCT TAATCCTCAA CACGTTGTCG

721 TCTTTGAGA GGAGGCCTAA AGGACAGGAG AAAAGGTCTT CAATCGTGG AAGAAAATTA
AGAAAACCTCT CCTCCGGATT TCCTGTCCTC TTTTCCAGAA GTTACGACCT TTCTTTAAT

781 AATGTTGTAT TAAATAGATC ACCAGCTAGT TTCAGAGTTA CCATGTACGT ATTCCACTAG
TTACAACATA ATTTATCTAG TGGTCGATCA AAGTCTCAAT GGTACATGCA TAAGGTGATC

FIG. 1 (CONTINUED).

841 CTGGGTTCTG TATTCAGTT CTTTCGATAC GGCTTAGGGT AATGTCAGTA CAGGAAAAAA
 GACCCAAGAC ATAAAGTCAA GAAAGCTATG CGGAATCCCA TTACAGTCAT GTCCCTTTTT

 901 ACTGTGCAAG TGAGCACCTG ATTCCGTTGC CTTGCTTAAC TCTAAAGCTC CATGTCCTGG
 TGACACGTTG ACCTCGTGGAC TAAGGCAACG GAACGAATTG AGATTTCGAG GTACAGGACC

 961 GCCTAAAATC GTATAAAATC TGGATTTTTT TTTTTTTTT TGCTCATATT CACATATGTA
 CGGATTTAG CATATTTAG ACCTAAAAAA AAAAAAAA ACGAGTATAA GTGTATACAT

 1021 AACCCAGAACAA TTCTATGTAC TACAAACCTG GTTTTTAAAAA AGGAACATATG TTGCTATGAA
 TTGGTCTTGT AAGATACATG ATGTTGGAC CAAAAATTTC TCCTTGATAC AACGATAACTT

 1081 TTAAACTTGTG GTCGTGCTGA TAGGACAGAC TGGATTTTTC ATATTTCTTA TTAAATTTTC
 AATTTGAACA CAGCACGACT ATCCTGTCTG ACCTAAAAAG TATAAGAAT AATTTAAAG

 1141 TGCCATTTAG AAGAAGAGAA CTACATTCACT GGTTTGGAAAG AGATAAACCT GAAAAGAAGA
 ACGGTAAATC TTCTTCTCTT GATGTAAGTA CCAAACCTTC TCTATTGGA CTTTCTTCT

 1201 GTGGCCTTAT CTTCACTTTA TCGATAAGTC AGTTTATTTG TTTCATTGTG TACATTTTA
 CACCGGAATA GAAGTGAAT AGCTATTCACT TCAAATAAAC AAAGTAAACAC ATGTAAGAAT

 1261 TATTCTCCTT TTGACATTAT AACTGTTGGC TTTCTAATC TTGTTAAATA TATCTATTTT
 ATAAGAGGAA AACTGTAATA TTGACAACCG AAAAGATTAG ACAATTAT ATAGATAAAA

 1321 TACCAAAGGT ATTTAATATT CTTTTTTATG ACAACTTAGA TCAACTATTT TTAGCTTGGT
 ATGGTTCCA TAAATTATAA GAAAAATAC TGTTGAATCT AGTTGATAAA AATCGAACCA

 1381 AAATTTTCTT AAACACAATT GTTATAGCCA GAGGAACAAA GATGATATAA AATATTGTTG
 TTTAAAAGA TTTGTGTTAA CAATATCGGT CTCTTGTTT CTACTATATT TTATAACAAAC

 1441 CTCTGACAAA AATACATGTA TTTCATTCTC GTATGGTGCT AGAGTTAGAT TAATCTGCAT
 GAGACTGTTT TTATGTACAT AAAGTAAAGAG CATAACCACGA TCTCAATCTA ATTAGACGTA

 1501 TTTAAAAAAC TGAATTGGAA TAGAATTGGT AAGTTGCAA GACTTTTGA AAATAATTAA
 AAATTTTTG ACTTAACCTT ATCTTAACCA TTCAACGTTT CTGAAAACCT TTTATTAATT

 1561 ATTATCATAT CTTCCATTCC TGTTATTGGA GATGAAAATA AAAAGCAACT TATGAAAGTA
 TAATAGTATA GAAGGTAAGG ACAATAACCT CTACTTTAT TTTCTGTTGA ATACTTTCAT

 1621 GACATTCAAGA TCCAGCCATT ACTAACCTAT TCCCTTTTTG GGGAAATCTG AGCCTAGCTC
 CTGTAAGTCT AGGTGGTAA TGATTGGATA AGGAAAAAAC CCCTTAGAC TCGGATCGAG

 1681 AGAAAAACAT AAAGCACCTT GAAAAAGACT TGGCAGCTTC CTGATAAACGC GTGCTGTGCT
 TCTTTTGTA TTTCGTGGAA CTTTTCTGA ACCGTCGAAG GACTATTGCG CACGACACGA

 1741 GTGCAGTAGG AACACATCCT ATTTATTGTG ATGTTGTGGT TTTATTATCT TAAACTCTGT
 CACGTCATCC TTGTGTAGGA TAAATAACAC TACAACACCA AAATAATAGA ATTTGAGACA

 1801 TCCATACACT TGTATAAATA CATGGATATT TTTATGTACA GAAGTATGTC TCTTAACCAG
 AGGTATGTGA ACATATTAT GTACCTATAA AAATACATGT CTTCATACAG AGAATTGGTC

 1861 TTCACTTATT GTACCTGG
 AAGTGAATAA CATGGACC

FIG. 2. Predicted VEGF-like protein encoded by Incyte contig of 8/12/98

1 MNIFLLNLLT EEVRLYSCTP RNFSVSIREE LKRTDTIFWP GCLLVKRCCG
51 NCACCLHNCN ECQCVPSKVT KKYHEVLQLR PKTGVRGLHK SLTDVALEHH
101 EECDCVCRGS TGG

FIG. 3. PCR primers for cloning VEGF-X

vegfX1	AAAATGTATGGATACAACCTTAC
vegfX2	GTTTGATGAAAGATTGGGCTTG
vegfX3	TTTCTAAAGGAAATCAAATTAG
vegfX4	GATAAGATTGTATCTGATG
vegfX5	GATGTCTCCTCTTCAG
vegfX6	GCACAACTCCTAATTCTG
vegfX7	AGCACCTGATTCCGTTGC
vegfX8	TAGTACATAGAACATGTTCTGG
vegfX9	AAGAGACATACTTCTGTAC
vegfX10	CCAGGTACAATAAGTGAAC TG

FIG. 4. Variants isolated by PCR (at 8/2/99, all cloned and sequenced at JRF)

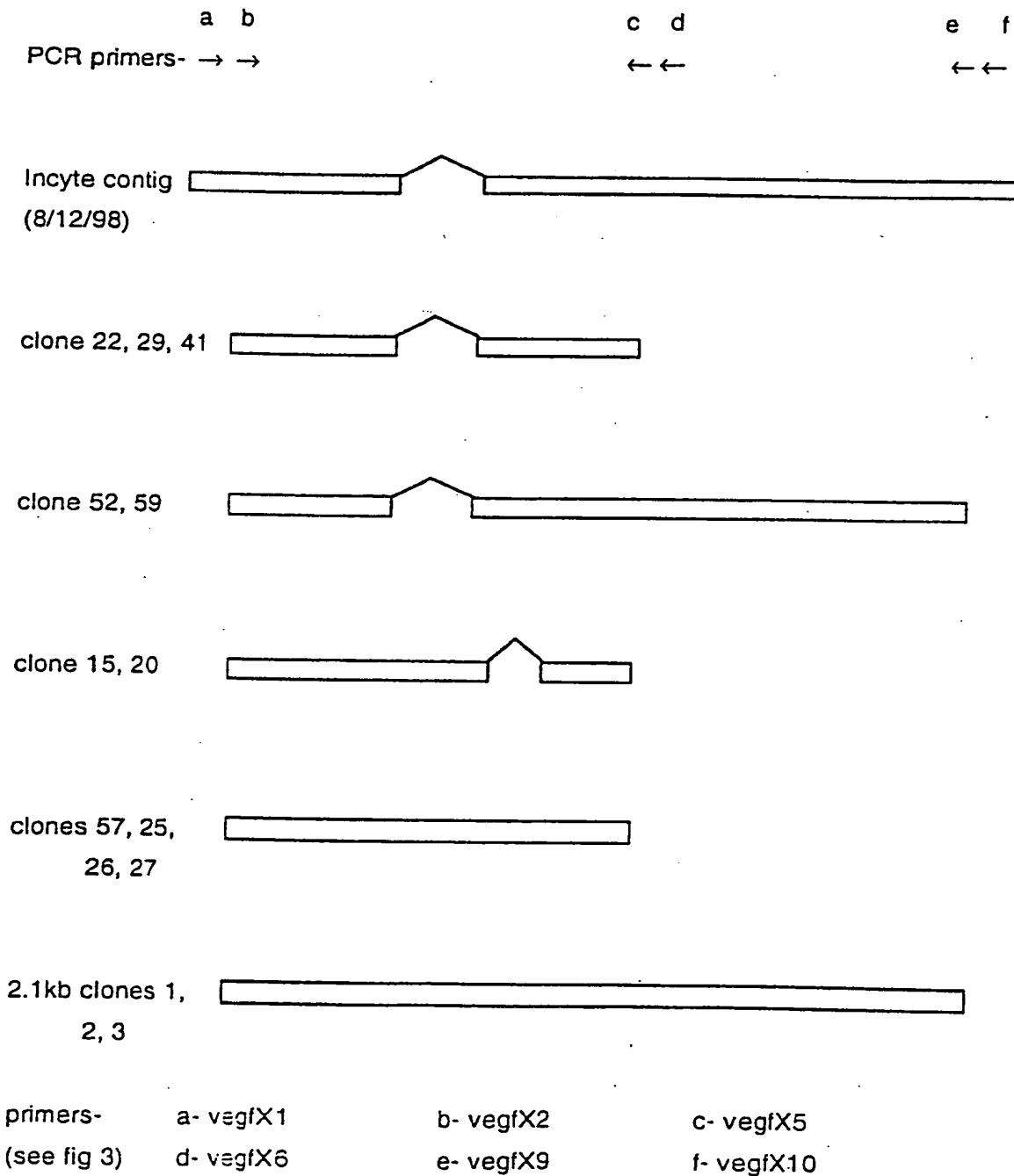


FIG. 5. VEGF-X 5' RACE primers

vegfX11	CCTTAGAAATCTGTTTCTGGTACAG
vegfX12	GGAAAATATTCAATCAGATAACAAATCTTATCC
vegfX13	GGTCCAGTGGCAAAGCTGAAGG
vegfX14	CTGGTTCAAGATATCGAATAAGGTCTTCC

FIG. 6. DNA sequence assembled from in-house clones and 5'RACE

1 TGCCAGAGCA GGTGGCGCT TCCACCCCAG TGCAGCCTTC CCCTGGCGGT GGTGAAAGAG
 ACGGTCTCGT CCACCCGCGA AGGTGGGTC ACGTCGGAAG GGGACCGCCA CCACCTTCTC
 61 ACTCGGGAGT CGCTGCTTCC AAAGTGCCCG CCGTGAGTGA GCTCTCACCC CAGTCAGCCA
 TGAGCCCTCA GCGACGAAGG TTTCACGGGC' GGCACTCACT CGAGAGTGGG GTCAGTCGGT
 +2 MetSerLeu PheGlyLeuLeu LeuLeuThr SerAlaLeu AlaGlyGlnArg GlnGlyTh
 121 AATGAGCCTC TTCTGGCTTC TCCTGCTGAC ATCTGCCCTG GCCGGCCAGA GACAGGGGAC
 TTACTCGGAG AAGCCCGAAG AGGACGACTG TAGACGGGAC CGGCCGGTCT CTGTCCCCCTG
 +2 rGlnAlaGlu SerAsnLeuSer SerLysPhe GlnPheSer SerAsnLysGlu GlnAsnG1
 181 TCAGGCGGAA TCCAACCTGA GTAGTAAATT CCAGTTTCC AGCAACAAGG AACAGAACGG
 AGTCCGCCTT AGGTTGGACT CATCATTTAA GGTCAAAAGG TCGTTGTCC TTGTCTTGCC
 +2 yValGlnAsp ProGlnHisGlu ArgIleIle ThrValSer ThrAsnGlySer IleHisSe
 241 AGTACAAGAT CCTCAGCATG AGAGAATTAT TACTGTGTCT ACTAATGGAA GTATTACAG
 TCATGTTCTA GGAGTCGTAC TCTCTTAATA ATGACACAGA TGATTACCTT CATAAGTGTG
 +2 rProArgPhe ProHisThrTyr ProArgAsn ThrValLeu ValTrpArgLeu ValAlaVa
 301 CCCAAGGTTT CCTCATACTT ATCCAAGAAA TACGGTCTTG GTATGGAGAT TAGTAGCAGT
 GGGTTCCAAA GGAGTATGAA TAGGTTCTT ATGCCAGAAC CATACTCTA ATCATCGTCA
 +2 lGluGluAsn ValTrpIleGln LeuThrPhe AspGluArg PheGlyLeuGlu AspProG1
 361 AGAGGAAAAT GTATGGATAC AACTTACGTT TGATGAAAGA TTTGGGCTTG AAGACCCAGA
 TCTCCTTTA CATACTATG TTGAATGCAA ACTACTTCT AAACCCGAAAC TTCTGGGTCT
 +2 uAspAspIle CysLysTyrAsp PheValGlu ValGluGlu ProSerAspGly ThrIleLe
 421 AGATGACATA TGCAAGTATG ATTTTGTAGA AGTTGAGGAA CCCAGTGATG GAACTATATT
 TCTACTGTAT ACGTTCATAC TAAAACATCT TCAACTCCTT GGGTCACTAC CTTGATATAA
 +2 uGlyArgTrp CysGlySerGly ThrValPro GlyLysGln IleSerLysGly AsnGlnII
 481 AGGGCGCTGG TGTGGTTCTG GTACTGTACC AGGAAAACAG ATTTCTAAAG GAAATCAAAT
 TCCCGCGACC ACACCAAGAC CATGACATGG TCCTTTGTC TAAAGATTC CTTTAGTTA
 +2 eArgIleArg PheValSerAsp GluTyrPhe ProSerGlu ProGlyPheCys IleHisTy
 541 TAGGATAAGA TTTGTATCTG ATGAATATT TCCTCTGAA CCAGGGTTCT GCATCCACTA
 ATCCTATTCT AAACATAGAC TACTTATAAA AGGAAGACTT GGTCCCAAGA CGTAGGTGAT
 +2 rAsnIleVal MetProGlnPhe ThrGluAla ValSerPro SerValLeuPro ProSerAl
 601 CAACATTGTC ATGCCACAAT TCACAGAAGC TGTGAGTCCT TCAGTGCTAC CCCCTTCAGC
 GTTGTAAACAG TACGGTGTAA AGTGTCTTCG ACACTCAGGA AGTCACGATG GGGGAAGTCG
 +2 aLeuProLeu AspLeuLeuAsn AsnAlaIle ThrAlaPhe SerThrLeuGlu AspLeuII
 661 TTTGCCACTG GACCTGCTTA ATAATGCTAT AACTGCCTTT AGTACCTTGG AAGACCTTAT

FIG. 6 (CONTINUED 1).

+2 eArgTyrLeu GluProGluArg TrpGlnLeu AspLeuGlu AspLeuTyrArg ProThrTr

721 TCGATATCTT GAACCAGAGA GATGGCAGTT GGACTTAGAA GATCTATATA GGCCAACTTG
AGCTATAGAA CTTGGTCTCT CTACCGTCAA CCTGAATCTT CTAGATATAT CGGGTTGAAC

+2 pGlnLeuLeu GlyLysAlaPhe ValPheGly ArgLysSer ArgValValAsp LeuAsnLe

781 GCAACTCTT GGCAAGGCTT TTGTTTTGG AAGAAAATCC AGAGTGGTGG ATCTGAACCT
CGTTGAAGAA CCGTTCCGAA AACAAAAACC TTCTTTAGG TCTCACCAACC TAGACTTGGG

+2 uLeuThrGlu GluValArgLeu TyrSerCys ThrProArg AsnPheSerVal SerIleAr

841 TCTAACAGAG GAGGTAAGAT TATACAGCTG CACACCTCGT AACTTCTCAG TGTCCATAAG
AGATTGTCTC CTCCATTCTA ATATGTCGAC GTGTGGAGCA TTGAAGAGTC ACAGGTATTG

+2 gGluGluLeu LysArgThrAsp ThrIlePhe TrpProGly CysLeuLeuVal LysArgCy

901 GGAAGAACTA AAGAGAACCG ATACCATTCTT CTGGCCAGGT TGTCTCCTGG TTAAACGCTG
CCTTCTTGAT TTCTCTTGGC TATGGTAAAA GACCGGTCCA ACAGAGGACC AATTTGCGAC

+2 sGlyGlyAsn CysAlaCysCys LeuHisAsn CysAsnGlu CysGlnCysVal ProSerLy

961 TGGTGGGAAC TGTGCCTGTT GTCTCCACAA TTGCAATGAA TGTCAATGTG TCCCAAGCAA
ACCACCCCTTG ACACGGACAA CAGAGGTGTT AACGTTACTT ACAGTTACAC AGGGTTCGTT

+2 sValThrLys LysTyrHisGlu ValLeuGln LeuArgPro LysThrGlyVal ArgGlyLe

1021 AGTTACTAAA AAATACCACG AGGTCCCTCA GTTGAGACCA AAGACCGGTG TCAGGGGATT
TCAATGATTT TTTATGGTGC TCCAGGAAGT CAACTCTGGT TTCTGGCCAC AGTCCCCTAA

+2 uHisLysSer LeuThrAspVal AlaLeuGlu HisHisGlu GluCysAspCys ValCysAr

1081 GCACAAATCA CTCACCGACG TGGCCCTGGA GCACCATGAG GAGTGTGACT GTGTGTGCAG
CGTGTCTAGT GAGTGGCTGC ACCGGGACCT CGTGGTACTC CTCACACTGA CACACACGTC

+2 gGlySerThr GlyGly

1141 AGGGAGCACA GGAGGATAGC CGCATCACCA CCAGCAGCTC TTGCCAGAG CTGTGCAGTG
TCCCTCGTGT CCTCCTATCG GCGTAGTGGT GGTCGTGAG AACGGGTCTC GACACGTAC

1201 CAGTGGCTGA TTCTATTAGA GAACGTATGC GTTATCTCCA TCCTTAATCT CAGTTGTTTG
GTCACCGACT AAGATAATCT CTTGCATACG CAATAGAGGT AGGAATTAGA GTCAACAAAC

1261 CTTCAAGGAC CTTTCATCTT CAGGATTAC AGTCATCT GAAAGAGGAG ACATCAAACA
GAAGTTCTG GAAAGTAGAA GTCCTAAATG TCACGTAAGA CTTCTCCTC TGTAGTTGT

1321 GAATTAGGAG TTGTGCAACA GCTCTTTGA GAGGAGGCCT AAAGGACAGG AGAAAAGGTC
CTTAATCCTC AACACGTGT CGAGAAAAGT CTCCTCCGGA TTTCTGTCC TCTTTCCAG

1381 TTCAATCGTG GAAAGAAAAT TAAATGTTGT ATTAAATAGA TCACCAAGCTA GTTTCAGAGT
AAGTTAGCAC CTTTCTTTA ATTACAACA TAATTATCT AGTGGTCGAT CAAAGTCTCA

1441 TACCATGTAC GTATTCCACT AGCTGGGTTTC TGTATTCAG TTCTTCGAT ACGGCTTAGG
ATGGTACATG CATAAGGTGA TCGACCCAAG ACATAAAGTC AAGAAAGCTA TGCCGAATCC

1501 GTAATGTCAG TACAGGAAAA AACTGTGCA AGTGAGCACC TGATTCCGTT GCCTTGCTTA

FIG. 6 (CONTINUED 2).

1561 ACTCTAAAGC TCCATGTCCT GGGCCTAAAA TCGTATAAAA TCTGGATTTT TTTTTTTTTA
 TGAGATTCG AGGTACAGGA CCCGGATTT AGCATATTAGACCTAAAA AAAAAAAA
 1621 TTTGCTCATA TTCACATATG TAAACCAGAA CATTCTATGT ACTACAAACC TGTTTTAA
 AAACGAGTAT AAGTGTATAC ATTTGGTCTT GTAAGATACA TGATGTTGG ACCAAAAATT
 1681 AAAGGAACTA TGTTGCTATG AATTAAACTT GTGTCGTGCT GATAGGACAG ACTGGATTT
 TTTCCTTGAT ACAACGATAC TTAATTGAA CACAGCACGA CTATCCTGTC TGACCTAAAA
 1741 TCATATTCT TATTAATTAGA TCTGCCATT AGAAGAACAG AACTACATTC ATGGTTGG
 AGTATAAAAGA ATAATTAAAGACCGTAAA TCTTCTTCTC TTGATGTAAG TACCAAACT
 1801 AGAGATAAAC CTGAAAAGAA GAGTGGCCTT ATCTTCACCTT TATCGATAAG CCAGTTATT
 TCTCTATTG GACTTTCTT CTCACCGGAA TAGAAGTGAA ATAGCTATTG GGTCAAATAA
 1861 TGTTTCATTG TGTACATTG TATATTCTCC TTTGACATT ATAACGTTG GCTTTCTAA
 ACAAAAGTAAC ACATGTAAA ATATAAGAGG AAAACTGTAA TATTGACAAC CGAAAAGATT
 1921 TCTTGTAAA TATATCTATT TTTACCAAAG GTATTTATA TTCTTTTTA TGACAACCTA
 AGAACAAATT ATATAGATAA AAATGGTTTC CATAAATTAT AAGAAAAAAT ACTGTTGAAT
 1981 GATCAACTAT TTTAGCTTG GTAAATTCTT CAAACACAA TTGTTATAGC CAGAGGAACA
 CTAGTTGATA AAAATCGAAC CATTAAAAA GATTGTTGTT ACAATATCG GTCTCCTTGT
 2041 AAGATGATAT AAAATATTGT TGCTCTGACA AAAATACATG TATTCATTG TCGTATGGTG
 TTCTACTATA TTTTATAACA ACGAGACTGT TTTATGTAC ATAAAGTAAG AGCATAACAC
 2101 CTAGAGTTAG ATTAATCTGC ATTTTAAAAA ACTGAATTGG AATAGAATTG GTAAGTTGCA
 GATCTCAATC TAATTAGACG TAAAATTCTT TGACTTAACC TTATCTTAAC CATTCAACGT
 2161 AAGACTTTT GAAAATAATT AAATTATCAT ATCTTCATT CCTGTTATTG GAGATGAAAA
 TTCTGAAAAA CTTTTATTAA TTTAATAGTA TAGAAGGTAA GGACAATAAC CTCTACTTT
 2221 TAAAAAGCAA CTTATGAAAG TAGACATTCA GATCCAGCCA TTACTAACCT ATTCTTTTT
 ATTTTCGTT GAATACTTTC ATCTGTAAGT CTAGGTCGGT AATGATTGGA TAAGGAAAA
 2281 TGGGAAATC TGAGCCTAGC TCAGAAAAAC ATAAAGCACC TTGAAAAAGA CTTGGCAGCT
 ACCCCTTAG ACTCGGATCG AGTCTTTTG TATTCGTGG AACTTTCT GAACCGTCGA
 2341 TCCTGATAAA GCGTGCTGTG CTGTGCAGTA GGAACACATC CTATTTATTG TGATGTTGTG
 AGGACTATTG CGCACGACAC GACACGTCAT CCTTGTGTAG GATAAATAAC ACTACAACAC
 2401 GTTTTATTAT CTTAAACTCT GTTCCATACA CTTGTATAAA TACATGGATA TTTTTATGTA
 CAAAATAATA GAATTGAGA CAAGGTATGT GAACATATTG ATGTACCTAT AAAAATACAT
 2461 CAGAAGTATG TCTCT
 GTCTTCATAC AGAGA

FIG. 7.

New Sequence + Incyte ESTs

1 ATTTGTTTAA ACCTTGGAA ACTGGTCAG GTCCAGGTT TGCTTGATC CTTTCAAAA
TAAACAAATT TGGAACCTT TGACCAAGTC CAGGTCCAAA ACGAAACTAG GAAAAGTTT

61 ACTGGAGACA CAGAAGAGGG CTTCTAGGAA AAAGTTTGG GATGGGATTA TGTGGAAACT
TGACCTCTGT GTCTTCTCCC GAAGATCCTT TTTCAAAACC CTACCTTAAT ACACCTTGA

121 ACCCTGCGAT TCTCTGCTGC CAGAGCAGGC TCGGCGCTTC CACCCAGTG CAGCCTTCCC
TGGGACGCTA AGAGACGACG GTCTCGTCCG AGCCGCGAAG GTGGGTCAC GTCGGAAGGG

181 CTGGCGGTGG TGAAAGAGAC TCGGGAGTCG CTGCTTCAA AGTGCCGCC GTGAGTGAGC
GACCGCCACC ACTTTCTCTG AGCCCTCAGC GACGAAGGTT TCACGGGCGG CACTCACTCG

+2 Met SerLeuPhe GlyLeuLeu LeuLeuThrSer AlaLeuAl

241 TCTCACCCCA GTCAGCCAA TGAGCCTCTT CGGGCTTCTC CTGCTGACAT CTGCCCTGGC
AGAGTGGGGT CAGTCGGTTT ACTCGGAGAA GCCCGAAGAG GACGACTGTA GACGGGACCG

+2 aGlyGlnArg GlnGlyThrGln AlaGluSer AsnLeuSer SerLysPheGln PheSerSe

301 CGGCCAGAGA CAGGGGACTC AGGCAGAATC CAACCTGAGT AGTAAATTCC AGTTTTCCAG
GCCGGTCTCT GTCCCTGAG TCCGCCTTAG GTTGGACTCA TCATTTAAGG TCAAAAGGTC

+2 rAsnLysGlu GlnTyrGlyVal GlnAspPro GlnHisGlu ArgIleIleThr ValSerTh

361 CAACAAGGAA CAGTACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA CTGTGTCTAC
GTTGTTCTT GTCATGCCTC ATGTTCTAGG AGTCGTACTC TCTTAATAAT GACACAGATG

+2 rAsnGlySer IleHisSerPro ArgPhePro HisThrTyr ProArgAsnThr ValLeuVa

421 TAATGGAAGT ATTACACAGCC CAAGGTTCC TCATACTTAT CCAAGAAATA CGGTCTTGGT
ATTACCTTCA TAAGTGTGG GTTCAAAGG AGTATGAATA GGTTCTTAT GCCAGAACCA

+2 lTrpArgLeu ValAlaValGlu GluAsnVal TrpIleGln LeuThrPheAsp GluArgPh

481 ATGGAGATTA GTAGCAGTAG AGGAAATGT ATGGATACAA CTTACGTTTG ATGAAAGATT
TACCTCTAAAT CATCGTCATC TCCTTTACA TACCTATGTT GAATGCAAAC TACTTTCTAA

+2 eGlyLeuGlu AspProGluAsp AspIleCys LysTyrAsp PheValGluVal GluGluPr

541 TGGGCTTGAAC GACCCAGAAG ATGACATATG CAAGTATGAT TTTGTAGAAG TTGAGGAACC
ACCCGAACCTT CTGGGCTTCTC TACTGTATAAC GTTCATACTA AAACATCTTC AACTCCTTGG

+2 oSerAspGly ThrIleLeuGly ArgTrpCys GlySerGly ThrValProGly LysGlnIl

601 CAGTGATGGA ACTATATTAG GGCGCTGGTG TGGTCTGGT ACTGTACCAAG GAAAACAGAT
GTCACTACCT TGATATAATC CCGCGACCAAC ACCAAGACCA TGACATGGTC CTTTTGTCTA

+2 eSerLysGly AsnGlnIleArg IleArgPhe ValSerAsp GluTyrPhePro SerGluPr

661 TTCTAAAGGA AATCAAATTA GGATAAGATT TGTATCTGAT GAATATTTC CTTCTGAACC
AAGATTTCCT TTAGTTAAT CCTATTCTAA ACATAGACTA CTTATAAAAG GAAGACTTGG

FIG. 7 (CONTINUED 1).

+2 oGlyPheCys IleHisTyrAsn IleValMet ProGlnPhe ThrGluAlaVal SerProSe

721 AGGGTTCTGC ATCCACTACA ACATTGTCA GGCACAAATTC ACAGAACGCTG TGAGTCCTTC
TCCCAAGACG TAGGTGATGT TGAAACAGTA CGGTGTTAAG TGTCTTCGAC ACTCAGGAAG

+2 rValLeuPro ProSerAlaLeu ProLeuAsp LeuLeuAsn AsnAlaIleThr AlaPheSe

781 AGTGCTACCC CCTTCAGCTT TGCCACTGGA CCTGCTTAAT AATGCTATAA CTGCCTTAG
TCACGATGGG GGAAGTCGAA ACGGTGACCT GGACGAATTAA TTACGATATT GACGGAAATC

+2 rThrLeuGlu AspLeuIleArg TyrLeuGlu ProGluArg TrpGlnLeuAsp LeuGluAs

841 TACCTTGAA GACCTTATTG GATATCTTGA ACCAGAGAGA TGGCAGTTGG ACTTAGAAGA
ATGGAACCTT CTGGAATAAG CTATAGAACT TGGTCTCTCT ACCGTCAACC TGAATCTTCT

+2 pLeuTyrArg ProThrTrpGln LeuLeuGly LysAlaPhe ValPheGlyArg LysSerAr

901 TCTATATAGG CCAACTTGGC AACTTCTTGG CAAGGCTTT GTTTTGAA GAAAATCCAG
AGATATATCC GGTTGAAACCG TTGAAGAACCC GTTCCGAAAA CAAAAACCTT CTTTTAGGTC

+2 gValValAsp LeuAsnLeuLeu ThrGluGlu ValArgLeu TyrSerCysThr ProArgAs

961 AGTGGTGGAT CTGAACTTTC TAACAGAGGA GGTAAGATTA TACAGCTGCA CACCTCGTAA
TCACCACCTA GACTTGGAAAG ATTGTCTCCT CCATTCTAAT ATGTCGACGT GTGGAGCATT

+2 nPheSerVal SerIleArgGlu GluLeuLys ArgThrAsp ThrIlePheTrp ProGlyCy

1021 CTTCTCAGTG TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTCT GGCCAGGTTG
GAAGAGTCAC AGGTATTCCC TTCTTGATTT CTCTTGGCTA TGGTAAAGA CCGGTCCAAC

+2 sLeuLeuVal LysArgCysGly GlyAsnCys AlaCysCys LeuHisAsnCys AsnGluCy

1081 TCTCCTGGTT AAACGCTGTG GTGGGAACTG TGCCCTGTTGT CTCCACAATT GCAATGAATG
AGAGGACCAA TTTGCCACAC CACCCCTTGAC ACGGACAACA GAGGTGTTAA CGTTACTTAC

+2 sGlnCysVal ProSerLysVal ThrLysLys TyrHisGlu ValLeuGlnLeu ArgProLy

1141 TCAATGTGTC CCAAGCAAAG TTACTAAAAA ATACCACGAG GTCCTTCAGT TGAGACCAAA
AGTTACACAG GGTTGTTTC AATGATTTT TATGGTGTCTC CAGGAAGTCA ACTCTGGTTT

+2 sThrGlyVal ArgGlyLeuHis LysSerLeu ThrAspVal AlaLeuGluHis HisGluGl

1201 GACCGGGTGTGTC AGGGGATTGCA ACAAACTCACT CACCGACGTG GCCCTGGAGC ACCATGAGGA
CTGGCCACAG TCCCCCTAACG TGTTTAGTGA GTGGCTGCAC CGGGACCTCG TGGTACTCCT

+2 uCysAspCys ValCysArgGly SerThrGly Gly

1261 GTGTGACTGT GTGTGCAGAG GGAGCACAGG AGGATAGCCG CATCACCACC AGCAGCTCTT
CACACTGACA CACACGTCTC CCTCGTGTCC TCCTATCGGC GTAGTGGTGG TCGTCGAGAA

1321 GCCCAGAGCT GTGCAGTGCA GTGGCTGATT CTATTAGAGA ACGTATGCGT TATCTCCATC
CGGGTCTCGA CACGTCACGT CACCGACTAA GATAATCTCT TGCATACGCA ATAGAGGTAG

1381 CTTAATCTCA GTTGTGGCT TCAAGGACCT TTCATCTTCA GGATTTACAG TGCATTCTGA
GAATTAGAGT CAACAAACGA AGTTCCCTGGA AAGTAGAAGT CCTAAATGTC ACGTAAGACT

FIG. 7 (CONTINUED 2).

1441 AAGAGGAGAC ATCAAACAGA ATTAGGAGTT GTGCAACAGC TCTTTGAGA GGAGGCCTAA
 TTCTCCTCTG TAGTTGTCT TAATCCTCAA CACGGTGTG AGAAAACCTCT CCTCCGGATT
 1501 AGGACAGGAG AAAAGGTCTT CAATCGTGGA AAGAAAATTA AATGTTGTAT TAAATAGATC
 TCCTGTCCTC TTTTCCAGAA GTTAGCACCT TTCTTTTAAT TTACAAACATA ATTTATCTAG
 1561 ACCAGCTAGT TTCAGAGTTA CCATGTACGT ATTCCACTAG CTGGGTTCTG TATTCAGTT
 TGGTCGATCA AAGTCTCAAT GGTACATGCA TAAGGTGATC GACCCAAGAC ATAAAGTCAA
 1621 CTTTCGATAC GGCTTAGGGT AATGTCAGTA CAGGAAAAAA ACTGTGCAAG TGAGCACCTG
 GAAAGCTATG CGGAATCCC TTACAGTCAT GTCCTTTTT TGACACGTT ACCTCGTGGAC
 1681 ATTCCGTTGC CTTGGCTTAA CTCTAAAGCT CCATGTCTG GGCCTAAAAT CGTATAAAAT
 TAAGGCAACG GAACCGAATT GAGATTCGA GGTACAGGAC CGGGATTTA GCATATTTA
 1741 CTGGATTTTT TTTTTTTTT TTGCGCATAT TCACATATGT AAACCAGAAC ATTCTATGTA
 GACCTAAAAA AAAAAAAA AACCGCGTATA AGTGTATACA TTTGGTCTG TAAGATACAT
 1801 CTACAAACCT GGTTTTAAA AAGGAACATAT GTTGCTATGA ATTAAACTTG TGTCATGCTG
 GATGTTGGA CCAAAATTT TTCTTGATA CAACGATACT TAATTGAAAC ACAGTACGGAC
 1861 ATAGGACAGA CTGGATTTTT CATATTCTT ATTAAAATTT CTGCCATTTA GAAGAAGAGA
 TATCCTGTCT GACCTAAAAA GTATAAAGAA TAATTTAAA GACGGTAAAT CTTCTCTCT
 1921 ACTACATTCA TGGTTGGAA GAGATAAACCC TGAAAAGAAC AGTGGCCTTA TCTTCACCTT
 TGATGTAAGT ACCAAACCTT CTCTATTGG ACTTTCTTC TCACCGGAAT AGAAGTGAAA
 1981 ATCGATAAGT CAGTTTATTG GTTCATTGT GTACATTTT ATATTCTCCT TTTGACATTA
 TAGCTATTCA GTCAAATAAA CAAAGTAACA CATGTAAAAA TATAAGAGGA AAACGTAAAT
 2041 TAACTGTTGG CTTTCTAAT CTTGTTAAAT ATATCTATT TTACCAAAGG TATTTAATAT
 ATTGACAACC GAAAAGATTA GAACAATTAA TATAGATAAA AATGGTTCC ATAAATTATA
 2101 TCTTTTTAT GACAACCTAG ATCAACTATT TTTAGCTTGG TAAATTTTC TAAACACAAT
 AGAAAAAATA CTGTTGAATC TAGTTGATAA AAATCGAACC ATTTAAAAG ATTTGTGTTA
 2161 TGTTATAGCC AGAGGAACAA AGATGATATA AAATATTGTT GCTCTGACAA AAATACATGT
 ACAATATCGG TCTCCTGTT TCTACTATAT TTTATAACAA CGAGACTGTT TTTATGTACA
 2221 ATTTCAATTCT CGTATGGTGC TAGAGTTAGA TTAATCTGCA TTTTAAAAAA CTGAATTGG
 TAAAGTAAGA GCATACCACG ATCTCAATCT AATTAGACGT AAAATTTTT GACTTAACCT
 2281 ATAGAATTGG TAAGTTGCAA AGACTTTTG AAAATAATTA AATTATCATA TCTTCCATTC
 TATCTTAACC ATTCAACGTT TCTGAAAAAC TTTTATTAAT TTAATAGTAT AGAAGGTAAG
 2341 CTGTTATTGG AGATGAAAAT AAAAAGCAAC TTATGAAAGT AGACATTCAG ATCCAGCCAT
 GACAATAACC TCTACTTTA TTTTCGTTG AATACTTTCA TCTGTAAGTC TAGTCGGTA
 2401 TACTAACCTA TTCTTTTTT GGGGAAATCT GAGCCTAGCT CAGAAAAACA TAAAGCACCT
 ATGATTGGAT AAGGAAAAAA CCCCTTACA CTCGGATCGA GTCTTTGT ATTTCGTGG
 2461 TGAAAAAGAC TTGGCAGCTT CCTGATAAAAG CGTGCTGTGC TGTGAGTAG GAACACATCC
 ACTTTCTG AACCGTCGAA GGACTATTTC GCACGACACG ACACGTCATC CTTGTGTTAGG
 2521 TATTTATTGT GATGTTGTGG TTTTATTATC TTAAACTCTG TTCCATACAC TTGTATAAAT
 ATAATAACA CTACAACACC AAAATAATAG AATTGAGAC AAGGTATGTG AACATATTTA

FIG. 7 (CONTINUED 3).

2581 ACATGGATAT TTTTATGTAC AGAAGTATGT CTCTTAACCA GTTCACTTAT TGTAACCTGG
TGTACCTATA AAAATACATG TCTTCATACA GAGAATTGGT CAAGTGAATA ACATGAGACC

2641 CAATTAAAAA GAAAATCAGT AAAATATTTT GCTTGTAAAAA TGCTTAATAT CGTGCGCTAGG
GTTAAATTTT CTTTAGTCA TTTTATAAAA CGAACATTAA ACGAATTATA GCACGGATCC

2701 TTATGTGGTG ACTATTTGAA TCAAAAATGT ATTGAATCAT CAAATAAAAG AATGTGGCTA
AATAACACCAC TGATAAAACTT AGTTTTACA TAACCTAGTA GTTTATTTC TTACACCGAT

2761 TTTTGGGGAG AAAATT
AAAACCCCTC TTTTAA

FIG. 8. Additional oligonucleotides used for amplification of entire coding region

5'-1 TTTGTTAACCTGGGAAACTGG
5'-2 GTCCAGGTTTGCTTGATCC

FIG. 9. DNA Sequence Of Clones 4 & 7, Identical Clones Containing The Entire Open Reading Frame

1 TTTGTTTAAA CCTTGGGAAA CTGGTCAGG TCCAGGTTT GCTTGATCC TTTCAAAAA
 AAACAAATTG GGAACCCTT GACCAAGTCC AGGTCCAAA CGAAACTAGG AAAAGTTTT

 61 CTGGAGACAC AGAACAGGGC TCTAGGAAA AGTTTGAT GGGATTATGT GGAAACTACC
 GACCTCTGTG TCTTCTCCCG AGATCCTTT TCAAAACCTA CCCTAATACA CCTTGATGG

 121 CTGCGATTCT CTGCTGCCAG AGCAGGCTCG GCGCTTCCAC CCCAGTGCAG CCTTCCCCCTG
 GACGCTAAGA GACGACGGTC TCGTCCGAGC CGCGAAGGTG GGGTCACGTC GGAAGGGGAC

 181 GCGGTGGTGA AAGAGACTCG GGAGTCGCTG CTTCAAAGT GCCC GCCGTG AGTGAGCTCT
 CGCCACCACT TTCTCTGAGC CCTCAGCGAC GAAGGTTCA CGGGCGGCAC TCACTCGAGA

 +2 MetSer LeuPheGly LeuLeuLeu LeuThrSerAla LeuAlaG1

 241 CACCCCAGTC AGCCAAATGA GCCTCTTCGG GCTTCTCCTG CTGACATCTG CCCTGGCCGG
 GTGGGGTCAG TCGGTTTACT CGGAGAACCC CGAAGAGGAC GACTGTAGAC GGGACCGGCC

 +2 yGlnArgGln GlyThrGlnAla GluSerAsn LeuSerSer LysPheGlnPhe SerSerAs

 301 CCAGAGACAG GGGACTCAGG CGGAATCCAA CCTGAGTAGT AAATTCCAGT TTTCCAGCAA
 GGTCTCTGTC CCCTGAGTCC GCCTTAGGTT GGACTCATCA TTAAAGGTCA AAAGGTCGTT

 +2 nLysGluGln AsnGlyValGln AspProGln HisGluArg IleIleThrVal SerThrAs

 361 CAAGGAACAG AACGGAGTAC AAGATCCTCA GCATGAGAGA ATTATTACTG TGTCTACTAA
 GTTCTTGTC TTGCTCTCATG TTCTAGGAGT CGTACTCTCT TAATAATGAC ACAGATGATT

 +2 nGlySerIle HisSerProArg PheProHis ThrTyrPro ArgAsnThrVal LeuValTr

 421 TGGAAGTATT CACAGCCCAA GTTCTCTCA TACTTATCCA AGAAATACGG TCTTGGTATG
 ACCTTCATAA GTGTCGGGT CCAGGAGT ATGAATAGGT TCTTATGCC AGAACCATAC

 +2 pArgLeuVal AlaValGluGlu AsnValTrp IleGlnLeu ThrPheAspGlu ArgPheG1

 481 GAGATTAGTA GCAGTAGAGG AAAATGTATG GATACAACCTT ACGTTGATG AAAGATTGG
 CTCTAACATCGT CATACTAACAC CTATGTTGAA TGCAAACATAC TTTCTAAAC

 +2 yLeuGluAsp ProGluAspAsp IleCysLys TyrAspPhe ValGluValGlu GluProSe

 541 GCTTGAAGAC CCAGAAGATG ACATATGCAA GTATGATTT GTAGAAGTTG AGGAACCCAG
 CGAACTCTG GGTCTTCTAC TGTATACGTT CATACTAAAA CATCTAACAC TCCTTGGGTC

 +2 rAspGlyThr IleLeuGlyArg TrpCysGly SerGlyThr ValProGlyLys GlnIleSe

 601 TGATGGAACAT ATATTAGGGC GCTGGTGTGG TTCTGGTACT GTACCAAGGAA AACAGATTTC
 ACTACCTTGA TATAATCCCG CGACCACACC AAGACCATGA CATGGTCCTT TTGTCTAAAG

 +2 rLysGlyAsn GlnIleArgIle ArgPheVal SerAspGlu TyrPheProSer GluProG1

 661 TAAAGGAAAT CAAATTAGGA TAAGATTGT ATCTGATGAA TATTTCCCTT CTGAACCAGG
 ATTTCCCTTA GTTTAACACA TAGACTACTT ATAAAAGGAA GACTTGGTCC

FIG. 9 (CONTINUED).

+2 yPheCysIle HisTyrAsnIle ValMetPro GlnPheThr GluAlaValSer ProSerVa

721 GTTCTGCATC CACTACAACA TTGTCATGCC ACAATTACA GAAGCTGTGA GTCTTCAGT
CAAGACGTAG GTGATGTTGT AACAGTACGG TGTTAAGTGT CTTCGACACT CAGGAAGTCA

+2 1LeuProPro SerAlaLeuPro LeuAspLeu LeuAsnAsn AlaIleThrAla PheSerTh

781 GCTACCCCCCT TCAGCTTG CACTGGACCT GCTTAATAAT GCTATAACTG CCTTTAGTAC
CGATGGGGGA AGTCGAAACG GTGACCTGGA CGAATTATTA CGATATTGAC GGAAATCATG

+2 rLeuGluAsp LeuIleArgTyr LeuGluPro GluArgTrp GlnLeuAspLeu GluAspLe

841 CTTGGAAGAC CTTATTCGAT ATCTTGAACC AGAGAGATGG CAGTTGGACT TAGAAGATCT
GAACCTCTG GAATAAGCTA TAGAACTTGG TCTCTCTACC GTCAACCTGA ATCTTCTAGA

+2 uTyrArgPro ThrTrpGlnLeu LeuGlyLys AlaPheVal PheGlyArgLys SerArgVa

901 ATATAGGCCA ACTTGGCAC ACCTGGCAA GGCTTTGTT TTTGGAAGAA AATCCAGAGT
TATATCCGCT TGAACCGTTG AAGAACCGTT CCGAAAACAA AAACCTCTT TTAGGTCTCA

+2 lValAspLeu AsnLeuLeuThr GluGluVal ArgLeuTyr SerCysThrPro ArgAsnPh

961 GGTGGATCTG AACCTCTAA CAGAGGAGGT AAGATTATAC AGCTGCACAC CTCGTAACCT
CCACCTAGAC TTGGAAGATT GTCTCCTCCA TTCTAATATG TCGACGTGTG GAGCATTGAA

+2 eSerValSer IleArgGluGlu LeuLysArg ThrAspThr IlePheTrpPro GlyCysLe

1021 CTCAGTGTCC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTTCTGGC CAGGTTGTCT
GAGTCACAGG TATTCCCTTC TTGATTTCTC TTGGCTATGG TAAAAGACCG GTCCAACAGA

+2 uLeuValLys ArgCysGlyGly AsnCysAla CysCysLeu HisAsnCysAsn GluCysGl

1081 CCTGGTTAAA CGCTGTGGTG GGAAGTGTGC CTGTTGTCTC CACAATTGCA ATGAATGTCA
GGACCAATTG GCGACACACC CCTTGACACG GACAACAGAG GTGTTAACGT TACTTACAGT

+2 nCysValPro SerLysValThr LysLysTyr HisGluVal LeuGlnLeuArg ProLysTh

1141 ATGTGTCCC AGCAAAGTTA CTAAAAAATA CCACGAGGTC CTTCAGTTGA GACCAAAGAC
TACACAGGGT TCGTTTCAAT GATTTTTAT GGTGCTCCAG GAAGTCAACT CTGGTTCTG

+2 rGlyValArg GlyLeuHisLys SerLeuThr AspValAla LeuGluHisHis GluGluCy

1201 CGGTGTCAAGG GGATTCACAA AATCACTCAC CGACGTGGCC CTGGAGCACC ATGAGGAGTG
GCCACAGTCC CCTAACGTGT TTAGTGAGTG GCTGCACCGG GACCTCGTGG TACTCCTCAC

+2 sAspCysVal CysArgGlySer ThrGlyGly

1261 TGACTGTGTG TGCAGAGGGA GCACAGGAGG ATAGCCGCAT CACCACCAGC AGCTCTGCC
ACTGACACAC ACGTCTCCCT CGTGTCTCC TATCGCGTA GTGGTGGTCG TCGAGAACGG

1321 CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCCTTAT CTCCATCCTT
GTCTCGACAC GTCACGTCAC CGACTAAGAT AATCTCTTGC ATACGCAATA GAGGTAGGAA

1381 AATCTCAGTT GTTGCTTCA AGGACCTTTC ATCTTCAGGA TTTACAGTGC ATTCTGAAAG
TTAGAGTCAA CAAACGAAGT TCCTGGAAAG TAGAAGTCCT AAATGTCACG TAAGACTTTC

1441 AGGAGACATC AAACAGAATT AGGAGTTGTG CAA
TCCTCTGTAG TTTGTCTAA TCCTCAACAC GTT

FIG. 10. Predicted Full-length Polypeptide Sequence

1 MSLFGLLLLT SALAGQRQGT QAESNLSSKF QFSSNKEQYG VQDPQHERII
51 TVSTNGSIHS PRFPHTYPRN TVLVWRLVAV EENVWIQLTF DERFGLEDPE
101 DDICKYDFVE VEEPSDGTIL GRWCGSGTVP GKQISKGNQI RIRFVSDEYF
151 PSEPGFCIHY NIVMPQFTEA VSPSVLPPSA LPLDLLNNAI TAFSTLEDLI
201 RYLEPERWQL DLEDLYRPTW QLLGKAFVFG RKSIVVVDLNL LTEEVRLYSC
251 TPRNFSVSIR EELKRTDTIF WPGCLLVKRC GGNACCLHN CNECQCVP SK
301 VTKKYHEVLQ LRPKTGVRGL HKSLTDVALE HHEECDCVCR GSTGG

FIG. 11

Alignment of VEGF-X with Other VEGFs

	*	20	*	40	*			
VEGF_HUMAN	:	-----				:	-	
PLGF_HUMAN	:	-----				:	-	
VEGB_HUMAN	:	-----				:	-	
VEGC_HUMAN	:	-----				:	-	
VEGD_HUMAN	:	-----				:	-	
990126vegx	:	MSLFGLLLTSALAGQRQGTQAESNLSSKFQFSSNKEQNGVQDPQHERII				:	50	
	60	*	80	*	100			
VEGF_HUMAN	:	-----				:	-	
PLGF_HUMAN	:	-----				:	-	
VEGB_HUMAN	:	-----				:	-	
VEGC_HUMAN	:	-----				:	-	
VEGD_HUMAN	:	-----				:	-	
990126vegx	:	TVSTNGSIHSPRFPHTYPRNTVLVWRLVAVEENVWIQLTFDERFGLEDPE				:	100	
	*	120	*	140	*			
VEGF_HUMAN	:	-----				:	-	
PLGF_HUMAN	:	-----				:	-	
VEGB_HUMAN	:	-----				:	-	
VEGC_HUMAN	:	-----MHLLGFFSVACSLAAALLPGPREAPAAAA				:	30	
VEGD_HUMAN	:	-----MYREWWVVNV				:	10	
990126vegx	:	DDICKYDFVEV--EPPSDGTTILGRWCSGTVPKGQISKGNQIRIRFVSDE				:	148	
	160	*	180	*	200			
VEGF_HUMAN	:	-----MN				:	2	
PLGF_HUMAN	:	-----MP				:	2	
VEGB_HUMAN	:	-----				:	-	
VEGC_HUMAN	:	-----AFESGLDLSDAEPDAGEATAYASKDLEEQLRSVSSVDELMTVLYPEYWKM				:	80	
VEGD_HUMAN	:	-----FMMLYVQLVQGSSNEHGPVKRSSQSTLERSEQIARAASSLEELLRITHSE				:	60	
990126vegx	:	-----YFPSEPGFCIHYNIVMPQFTEAVSPSVLPPSALPLDLLNNAITAFSTLED				:	198	
	*	220	*	240	*			
VEGF_HUMAN	:	-----VYQRSY				:	51	
PLGF_HUMAN	:	-----VMRLFPCFLQLLAGLALPAVPPQQWALSAGNGSSEVEVVPFQE-VWGRSY				:	51	
VEGB_HUMAN	:	-----MSPLLRRLLLAAALLQLAPAQAPVSQPDAPGHQRKVVSID-VYTRAT				:	46	
VEGC_HUMAN	:	-----YKCQLRKGGWQHNREQANLNSRTEETIKFAAHYNTETILKSIDNEWRK-TQ				:	130	
VEGD_HUMAN	:	-----DWKLWRCRLRLKSFTSMSRSASHRSTRFAATFYDIETLKVIDEEWQRTQ				:	110	
990126vegx	:	-----LIRYLEPERWQLDLEDLYRPTWQLLGKAFVGRKSRVVDLNLLTEEVRLY				:	248	
	260	*	280	*	300			
VEGF_HUMAN	:	CHPIETLVDFQEYPDEIEYIFKPSCVPLMRCGG	CCND	--EGLECV	VP	:	96	
PLGF_HUMAN	:	CRALERLVDVVSEYPSEVEHMFS	CCGD	--ENLHC	VP	:	96	
VEGB_HUMAN	:	CQPREVVVPLTVELMGTVAQQLV	CCPD	--DGLE	CV	:	91	
VEGC_HUMAN	:	CMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGG	CCNS	--EGLQC	MN	:	175	
VEGD_HUMAN	:	CSPRETCVEVAS	CCNE	--ESLIC	MN	:	155	
990126vegx	:	ELGKSTNTFFKPPCVNVFR	CCCL	HNC	ECOC	CV	:	298

FIG. 11 (CONTINUED).

	* 320 * 340 *
VEGF_HUMAN : TEESNITM QIMR I KPHQG -----QHIGEMSFLQHNKCECRPKKDRARQE K : 141	
PLGF_HUMAN : VETANVTM QOLL KIRSGDR-----PSYVELTFSQHVRCECRPLREKMKPER : 141	
VEGB_HUMAN : TGQHQVRM QILM TRYPS-----SQLGEMSLEEH SQCECRPKKKD SAVKP : 135	
VEGC_HUMAN : TSTS YL SKTLFEITVPLSQG---PKPV TISF ANHTSCROMSKLDVYRQVH : 222	
VEGD_HUMAN : TSTS YISKOL FEISVPLTSV---PELVPVKVANHTGCKCLPTAPRHPYSI : 202	
990126vegx : SKVTKKYHEVL QLR PKTGVRLHKSLTDVALEHH ECD CVCRGSTGG--- : 345	
	360 * 380 * 400
VEGF_HUMAN : KSVRGKG GQKR RKKSRYS WSV P----- : 166	
PLGF_HUMAN : ----- : -	
VEGB_HUMAN : DS PR ----- : 139	
VEGC_HUMAN : S IIRRSLP ATLPQCQAANKTCPTNYMWNNHICRCLAQEDFMFSSDAGDD S : 272	
VEGD_HUMAN : IRRS IQI PEEDRC SHSK KLC PIDML WDSNKCKV LQ ENPLAGT----- : 246	
990126vegx : ----- : -	
	* 420 * 440 *
VEGF_HUMAN : ----- : -	
PLGF_HUMAN : ----- : -	
VEGB_HUMAN : ----- : -	
VEGC_HUMAN : TDGFHD ICGP NKE LD EETCQCVCRAGLRPASC GPH KELDRNSC QC V C KNK : 322	
VEGD_HUMAN : ----- : EDHSHL QEPAL CGP : 260	
990126vegx : ----- : -	
	460 * 480 * 500
VEGF_HUMAN : ----- : CGPCSER RKHL FVQDPQTCKC-SCKNTDSRCKARQ LELN ER : 206	
PLGF_HUMAN : ----- : CGDAVPRR----- : 149	
VEGB_HUMAN : ----- : PLCPRCTQHHQRPDPRTCR CR RRSFLRCQGRGLELNPD : 179	
VEGC_HUMAN : LFP SQCGANREFD ENTCQCVC KRT CPRNQPLNP PGKACE CTESPQKCLLK : 372	
VEGD_HUMAN : HMMFD EDRCE CVCKTPCP KDLI QHP KN CSCFECKESLETCC QKH LFHPD : 310	
990126vegx : ----- : -	
	* 520 * 540 *
VEGF_HUMAN : TCRC DKP RR----- : 215	
PLGF_HUMAN : ----- : -	
VEGB_HUMAN : TCRC RKL RR----- : 188	
VEGC_HUMAN : GKKFH HQTCS CYRRPCTNRQ KACE PGFSYSEEV CRCV PSYWK PQMS --- : 419	
VEGD_HUMAN : TCS CEDRCP FHTRPCASG KTACAK CRFP KEKRAA QGPHSRKNP----- : 354	
990126vegx : ----- : -	

FIG. 12.

Variant Polypeptide Sequences

FL_seq :	MSLFGLLLLTSALAGQRQGTQAESNLSSKFQFSSNKEQNGVQDPQHERII	*	20	*	40	*	
clone41 :	MSLFGLLLLTSALAGQRQGTQAESNLSSKFQFSSNKEQNGVQDPQHERII						: 50
clone20 :	MSLFGLLLLTSALAGQRQGTQAESNLSSKFQFSSNKEQNGVQDPQHERII						: 50
FL_seq :	TVSTNGSIHSPRFPTYPRTNTVLWWRLVAVEENVWIQLTFDERFGLEDPE	*	60	*	80	*	100
clone41 :	TVSTNGSIHSPRFPTYPRTNTVLWWRLVAVEENVWIQLTFDERFGLEDPE						: 100
clone20 :	TVSTNGSIHSPRFPTYPRTNTVLWWRLVAVEENVWIQLTFDERFGLEDPE						: 100
FL_seq :	DDICKYDFVEVEEPESDGTTILGRWCNSGTVPKGQISKGNQIRIRFVSDEYF	*	120	*	140	*	
clone41 :	DDICKYDFVEVEEPESDGTTILGRWCNSGTVPKGQISKGNQIRIRFVSDEYF						: 150
clone20 :	DDICKYDFVEVEEPESDGTTILGRWCNSGTVPKGQISKGNQIRIRFVSDEYF						: 150
FL_seq :	PSEPGFCIHYNIVMPQFTEAVSPSVLPPSALPLDLLNNAITAFSTLEDLI	*	160	*	180	*	200
clone41 :	PSEPSNRGGKIIQLHTS-----						: 200
clone20 :	PSEPGFCIHYNIVMPQFTEAVSPSVLPPSALPLDLLNNAITAFSTLEDLI						: 167
FL_seq :	RYLEPERWQLDLEDLYRPTWQLLGKAFVFGRKSRVVDLNLLTEEVRLYSC	*	220	*	240	*	
clone41 :	-----						: 250
clone20 :	RYLEPERWQLDLEDLYRPTWQLLGKAFVFGRKSRVVDLNLLTE-----						: 243
FL_seq :	TPRNFSVSIREELKRTDTIFWPGCCLVKRCGGNCACCLHNCNECQCVP SK	*	260	*	280	*	300
clone41 :	-----						: 300
clone20 :	-----						: -
FL_seq :	VTKKYHEVLQLRPKTGVRGLHKSLTDVALEHHEECDCVCRGSTGG	*	320	*	340	*	
clone41 :	-----						: 345
clone20 :	-----EVLQLRPKTGVRGLHKSLTDVALEHHEECDCVCRGSTGG						: 282

*FIG. 13. Primers for Expression of VEGF-X**E.coli expression of domain-*

vegx-6	AATTGGATCCGAGAGTGGTGGATCTGAACC
vegx-7	AATTGGATCCGGGAAGAAAATCCAGAGTGG
vegx-8	GGTTGAATTCAATTATTTTAGTAACTTGCTGGGACAC
vegX-9	AATTGAATTCAATTATCCTCCTGTGCTCCCTC

Baculovirus/insect cell expression of full-length protein-

vegbac1	AATTGGATCCGGAGTCTCACCATCACCAACCATCATGAATCCAACCTGAGTAGTAAATT C
vegbac2	AATTGAATTCGCTATCCTCCTGTGCTCCCTCTGC

FIG. 14.

>3993180H1 LUNGNON03 INCYTE
 CACAAATCACTCACCGACGTGGCCCTGGAGCACCAGGNGTGTGACTGTGTGAGAGGGAGCACAGGAGGATAGCC
 GCATCACCAACCAGCAGCTCTGCCAGAGCTGTGCAGTCAGTGGTGTGATTCTATTAGAGAACGTTACAGTGCATTCTGAAAGAGGAGACATCAAACAG
 AATTAGGAGTTGTGCAACAGCTCTTGAGAGGAGGCTAAAGGACAGGAGAANAGGTCTT
>3510192H1 CONCNOT01 INCYTE
 TGCAGTGCAGTGGCTGATTCTATTAGAGAACGCTGTGCGTTATCTCCATCCTTAATCTCAGTTGTTGCTCAAGGACCTT
 TCATCTTCAGGATTACAGTCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTGAGAG
 GAGGCCTAAAGGACAGGAGAAAAGGCTTCATCGTGGAAAGAAAATTAAATGTTGATTAAATAGATCACAGCTAGTT
 TCAGAGTTACCATGTACGTATTCCACTAGCTGGTTCTGTATT
>2559870H1 ADRETUT01 INCYTE
 CACGGAGGTCTTCAGTTGAGACCAAAGACCGGTGTCAGGGGATTGACAACAAACTCACCGACGTGGCCCTGGAGCACCA
 TGAGGAGTGTGACTGTGTGTCAGAGGGACAGGGGGATAGCCGATCACCACAGCAGCTCTGCCAGAGCTGTG
 AGTGCAGTGGCTGATTCTATTAGAGAACGCTATGCGTTATCTCCATCCTTAATCTCAGTTGTTGCTCAAGGACCTTCA
 TCTTCAGGATTACAGTCATTCTGAAAGAGGAGA
>3979767H1 LUNGTUT08 INCYTE
 GGAGGATAGCCGCATCACCACCAAGCAGCTCTGCCAGAGCTGTGCAAGTGGCTGATTCTATTAGAGAACGTTATG
 GTTATCTCCATCCTTAATCTCAGTTGTTGCTCAAGGACCTTCACTCTTCAGGATTACAGTCATTCTGAAAGAGGAG
 ACATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTGAGAGGAGGCTAAAGGACAGGAGAAAAGGTCTTCATCGT
 GAAAGAANATTAAATGTTGATTAAATAGACACCAGCT
>3980011H1 LUNGTUT08 INCYTE
 GGAGGATAGCCGCATCACCACCAAGCAGCTCTGCCAGAGCTGTGCAAGTGGCTGATTCTATTAGAGAACGTTATG
 GTTATCTCCATCCTTAATCTCAGTTGTTGCTCAAGGACCTTCACTCTTCAGGATTACAGTCATTCTGAAAGAGGAGA
 CATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTGAGAGGAGGCTAAAGGACAGGAGAAAAGGTCTTCATCGTGG
 AAAGAAAATTAAATGTTGATTAAATAGATCACCA
>4825396H1 BLADDIT01 INCYTE
 GAGAACCGATAACCATTCTGGCCAGGGTGTCTCTGGTTAACAGCTGTGGTGGAACTGTGCCTGTTGCTCCACAATT
 GCAATGAATGTCATGTGCCCCAACAGAAAGTTACTAAAAAAATACCACGAGGTCTTCAGTTGAGACCAAAAGACGGTG
 AGGGATTGCACAAATCACTCACCGACGTGGCCCTGGAGCACATGAGGAGTGTGACTGTGTGCAAGAGGGAGCACAGG
 AGGATAGCCGCATCACCACCA
>3073703H1 BONEUNT01 INCYTE
 AGAAAATCCAGAGTGGTGTGACTGTGAAACCTTCTAACAGAGGAGGTAAGATTATAACAGCTGCACACCTCGTAACCTCTCAGT
 GTCCATAAGGAGAACACTAAAGAGAACCGATACCAATTCTGGCCAGGGTGTCTCTGGTTAACGCTGTGGTGGAACT
 GTGCCCTGGTGTCTCCACAAATTGCAATGAATGTCATGTGCCCCAACAGAAAGTTACTAAAAAAATACCACGAGGTCTTCAG
 TTGAGACCAAAGACCGGTGTCAGGGATTGACAACAAATCA
>1302516H1 PLACNOT02 INCYTE
 AGGAAATCAAATTAGGATAAGATTGTATCTGATGAATATTTCTCTGAACCTCTAACAGAGGAGGTAAGATTATAC
 AGCTGCACACCTCGTAACCTCTCAGTGTCCATAAGGAGAACACTAAAGAGAACCGATACCATTCTGCCAGGTGTCT
 CCTGGTTAACGCTGTGGTGGAACTGTGCCTGTTGCTCTCCACAATTGCAATGAATGTCATGTGCTCCAAGCAAAGTT
 ACTAAAAAAATACCACGAGGTCC
>3684109H1 HEEANOT01 INCYTE
 ATTTCATCTTCAGGATTACAGTCATTCTGAAANAGGAGAAAATCAAACANAATTAGGAGTTGTGCAACAGCTCTTTGA
 GAGGAGGCCTAAAGGACAGGAGAAAAGGCTTCATCGTGGAAANAAAATTAAATGTTGATTAAATAGATCACCGACT
 GTTTCAGAGTTACCATGTACGTATTCCACTAGCTGGGTTCTGTATTTCAGTTCTCGATACGGCTTAGGTAATGTCAG
 TACAGGAAAAAAACTGTGCAAGTGAGCACCTGATTCCGTGCTTGCCTTGCCT
>4713188H1 BRAHCT01 INCYTE
 CAAAGTTACTAAAAAAATACCACGAGGTCTTCAGTTGAGACCAAAGACCGGTGTCAGGGGATTGCAACAAATCACTCACCG
 ACCTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGCAAGGGAGCACAGGAGGATAGCCGCATACCACCAAGCAG
 CTCTGCCAGAGCTGTGCAAGTGGCTGATTCTATTAGAGAACGTTATGCGTTATCTCCATCCTTAATCTCAGTTGT
 TTGCT
>458823H1 KERANOT01 INCYTE
 ANGAGTTGCCAGAGCTGTGCAGTCAGTGGTGTGATTCTATTAGAGAACGTTATGCGTTATCTCCATCCTTAATCTCAGT
 GTTGTNTCAAGGACCTTCACTCTCAGGATTACAGTCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTG
 CAACAGCTTTTGAGAGGAGGCTAAAGGNACGGAGAAAAGGTCTCAATCGTGGAAAGAAAATTAAATGTTGATTAA
 ATAGATC
>1303909H1 PLACNOT02 INCYTE
 AGGAAATCAAATTAGGATAAGATTGTATCTGATGAATATTTCTCTGAACCTCTAACAGAGGAGGTAAGATTATAC
 AGCTGCACACCTCGTAACCTCTCAGTGTCCATAAGGAGAACACTAAAGAGAACCGATACCATTCTGCCAGGTGTCT
 CCTGGTTAACGCTGTGGTGGAACTGTGCCTGTTGCTCCACAATTGCAATGAATGTCATGTGCTCCAAG

FIG. 14 (CONTINUED).

>2739211H1 OVARNOT09 INCYTE
 GTGCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTGCAACAGCTCTTGTGAGAGGAGGCCTAAAGGACAGGA
 GAAAAGGTCTTCATCGTGGAAAGAAAATTAAATGTTGTATTAAATAGATCACCGTAGTTCAGAGTTACCATGTACG
 TATTCCACTAGCTGGTTCTGTATTCAGTTCTCGATACGGCTTAGGTAATGTCAGTACAGGAAAAACTGTGCAA
 GTGAGCACCTGAT

>3325591H1 PTHYNOT03 INCYTE
 TGCAACAGCTCTTGAGAGGAGGCCTAAAGGACAGGAGAAAAGGTCTCAATCGTGGAAAGAAAATTAAATGTTGTATT
 AAATAGATCACCACTAGTTCAGAGTTACCATGTACGTATTCCACTAGCTGGTTCTGTATTTCAGTTCTTCGATACG
 GCTTAGGTAATGTCAGTACAGGAAAAACTGTGCAAGTGAGCACCTGATTCGTTGCCCTGCTTAACCCTAAAGCNCC
 ATGTCNNGGCNAAAANCAGAAAAAT

>3733565H1 SMCCNOS01 INCYTE
 CCTTAATCTCAGTTGCTTCAGGACCTTCATCTCAGGATTACAGTCATTCTGNAAGANGAGACATCAAACAG
 AATTAGGNGTTGTGCAAAGCTTTGAGAGGAGGCCTAAAGGACAGGAGAAAAGGTCTNCAATCGTGGAAAGNAAATT
 AAATGTTGTATNAAATNGATCACCACTAGTTCAGAGTTACCATGTACGTATTCCACTAGCTGGNCNGTATTCACTCT
 TTCGAAACGGCTTAGGTAATGTCAGTACAGGAAAAACTGTGAGTCAG

>3554223H1 SYNONOT01 INCYTE
 ATAAAATAGATCACCACTAGTTCAGAGTTACCATGTACGTATTCCACTAGCTGGTTCTGTATTTCAGTTCTTCGAT
 ACGGCTTAGGTAATGTCAGTACAGGAAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCCTGGCTTAACTCTAAAG
 CTCCATGTCCTGGCCTAAATCGTATAAAATCTGGATTTNTTTTTGCGCATATTCACATATGTAACCAGN
 ACATTCTATGTACNACAAACCTGGTTTTAAAAAGGAAC

>4507477H1 OVARTDT01 INCYTE
 GGCTAGTTTCAGAGTTACCATGTACGTATTCCACTAGCTGGTTCTGTATTTCAGTTCTTCGATACGGCTTAGGTAAT
 GTCAGTACAGGAAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCCTGGCTTAACTCTAAAGCTCCATGTCCTGGCC
 TAAAATCGTATAAAATCTGGA

>4163378H1 BRSTNOT32 INCYTE
 AATAGATCACCACTAGTTCAGAGTTACCATGTACGTATTCCACTAGCTGGNTCTGTATTTCAGTTCTTCGATACG
 GCTTAGGTAATGTCAGTACAGGAAAAAAAGCTGTGCAAGTGAGCACCTGATTCCGTTGCCCTGGCTTAACTCTAAAGCTCC
 ATGTCCTGGCCTAAATCGTATA

FIG. 15.

>2054675H1 BEPINOT01 INCYTE
 AAAGGAACATATGTCATGAATTAAACTTGTGCGTGTGATAGGACAGACTGGATTTCATATTCTTATTAAAATT
 TCTGCCATTAGAAGAAGAGAACTACATTCACTGTTGGAAGAGATAAACCTGAAAAGAAGAGTGGCCTTATCTCACTT
 TATCGATAAGTCAGTTATTGTTCATGTGACATTTTATATTCTCCTTGACATTATAACTGTTGGCTTTCTAA
 TCTTGTAAATATCTATTACCAAAGGTATTAATATTCTTTTA
>3993180H1 LUNGNON03 INCYTE
 CACAATCACTCACCGACGTGGCCCTGGAGCACCATGAGGNGTGTGACTGTGTGCAGAGGGAGCACAGGAGGATAGCC
 GCATCACCACCAAGCAGCTCTGCCAGAGCTGTGCACTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCAT
 CCTTAATCTCAGTTGTTGCTCAAGGACCTTCATCTCAGGATTACAGTCATTCTGAAAGAGGAGACATCAAACAG
 AATTAGGAGTTGTGCAACAGCTTTGAGAGGAGCTAAAGGACAGGAGAANAGGTCTT
>3510192H1 CONCN0T01 INCYTE
 TGCAGTGCAGTGGCTGATTCTATTAGAGAACGTATGCTTATCTCCATCCTTAATCTCAGTTGTTGCTTCAAGGACCTT
 TCATCTCAGGATTACAGTCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTGCAACAGCTTTGAGAG
 GAGGCCAAAGGACAGGAGAAAGGCTTCATCTGAAAGAGAAATTAATGTTGATTAAATAGATCACCAGCTAGTT
 TCAGAGTTACCATGTACGTATTCCACTAGCTGGTTCTGTATT
>4164633H1 BRSTNOT32 INCYTE
 CTTGTTAAATATCTATTACCAAAGGTATTAATATTCTTANTTATGACAACCTAGATCAACTATTTTAGCTTG
 GTAAATTCTAAACACAATTGTTATGCCAGAGAACAAAGATGATATAAAATATTGTCCTGACAAAAATACATG
 TATTTCATTCTGTATGGTCTAGAGTTAGATTAATCTGCACTTTAAAAACTGAATTGGAATAGAATTGGTAAGTGCA
 AAGACTTTGANAATAATTAATTATCATATCTCCTGTTATTGGGGAGAAAAT
>2559870H1 ADRETUT01 INCYTE
 CACGAGGTCCTTCAGTTGAGACCAAAGACCGGTGTCAGGGGATTGCCACAAATCACTCACCGACGTGGCCCTGGAGCACCA
 TGAGGAGTGTGACTGTGTGCAAGGGAGCACAGGGGATAGCCGCATCACCACAGCAGCTTGTGCCCAGAGCTGTG
 AGTGCAGTGGCTGATTCTATTAGAGAACGTATGCTTATCTCCATCCTTAATCTCAGTTGTTGCTTCAAGGACCTTCA
 TCTCAGGATTACAGTCATTCTGAAAGAGGAGA
>3817470H1 BONSTUT01 INCYTE
 TTAAAAAGGAACATATGTCATGAATTAAACTTGTGTCATGCTGATAGGACAGACTGGATTTCATATTCTTATTAA
 AATTCTGCCATTAGAAGAAGAGAACTACATTCACTGGTTGGAAGAGATAAACCTGAAAAGAAGAGTGGCCTTATCTC
 ACTTATCGATAAGTCAGTTATTGTTCATGTGACATTTTATATTCTCCTTTGACATTATAACTGTTGGCTTTC
 TAATCTGTTAAATATCTATTACCAAAGGTATTAATATTCTT
>3979767H1 LUNGUT08 INCYTE
 GGAGGATAGCCGCATCACCAACAGCAGCTTGTGCCCAGAGCTGTGCACTGGCTGATTCTATTAGAGAACGTATGC
 GTTATCTCCATCCTTAATCTCAGTTGTTGCTTCAGGACCTTCATCTCAGGATTACAGTCATTCTGAAAGAGGAG
 ACATCAAACAGAATTAGGAGTTGTGCAACAGCTTTGAGAGGAGGCTAAAGGACAGGAGAAAAGTCTCAATCGTG
 GAAAGAANATTAAATGTTGATTAAATAGACACCAGCT
>3980011H1 LUNGUT08 INCYTE
 GGAGGATAGCCGCATCACCAACAGCAGCTTGTGCCCAGAGCTGTGCACTGGCTGATTCTATTAGAGAACGTATGC
 GTTATCTCCATCCTTAATCTCAGTTGTTGCTTCAGGACCTTCATCTCAGGATTACAGTCATTCTGAAAGAGGAGA
 CATCAAACAGAATTAGGAGTTGTGCAACAGCTTTGAGAGGAGGCTAAAGGACAGGAGAAAAGTCTCAATCGTGG
 AAAGAAAATTAAATGTTGATTAAATAGATCACCA
>4825396H1 BLADDIT01 INCYTE
 GAGAACCGATACCATTCTGCCAGGGTGTCTCTGGTAAACGCTGTGGTGGAACTGTGCCTGTTGCTCCACAATT
 GCAATGAATGTCAATGTGCTCAAGCAAAGTTACTAAAAAATACCACGAGGTCTTCAGTTGAGAGACCAAAGCCGGTGTG
 AGGGGATTGCAAAATCACTCACCGACGTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGCAAGAGGAGCACAGG
 AGGATAGCCGCATCACCAACCA
>3073703H1 BONEUNT01 INCYTE
 AGAAAATCCAGAGTGGTGGATCTGAACCTTCTAACAGAGGAGGTAAGATTATAACAGCTGCACACCTCGTAACCTCTCAGT
 GTCCATAAGGGAAAGAACTAAAGAGAACCGATACCATTCTGCCAGGGTGTCTCTGGTAAACGCTGTGGTGGAACT
 GTGCCGTGTTGCTCCACAATTGCAATGAATGTCAATGTGCTCCAAAGCAAAGTTACTAAAAAATACCACGAGGTCTTCAG
 TTGAGACCAAAGACCGGTGTCAGGGATTGCAACAAATCA
>862169H1 BRAITUT03 INCYTE
 AGATGATATAAAATATTGTCATGACAAAAATACATGTTGCTGATGGTCTAGAGTTAGATTAATCTGCA
 TTTTAAAAAAACTGAATTGGAATAGAATTGGAAGTTGCAAAAGACTTTGAAAATAATTAAATTATCATATCTCATT
 CTGTTATTGGAGATGAAAATAAAAGCAACTTATGAAAGTAGACATTCAACCTATTCTCCTTT
 GGGGAAATCTGAGCCTAGC
>4201385H1 BRAITUT29 INCYTE
 TTTTAAAAAGGAACATATGTCATGAATTAAACCTTGTGCGTGTGATAGGACAGACTGGATTTCATATTCTTAT
 TAAAATTCTGCCATTAGAAGAAGAGAACTACATTCACTGGTTGGAAGAGATAAACCTGAAAAGAAGAGTGGCCTATCT
 TCACATTATCGATAAGTCAGTTATTGTTCATGTGACATTTTATATTCTCCTTGACATATAACTGTTGGCTTT

FIG. 15 (CONTINUED 1).

CTAATCTGTTAAATATATCTATTTACCAAAGGTATTAATAT
 >1302516H1 PLACNOT02 INCYTE
 AGGAAATCAAATTAGGATAAGATTGTATCTGATGAATATTTCTCTGAACCTCTAACAGAGGAGGTAAAGATTATAC
 AGCTGCACACCTCGTAACCTCTCAGTGTCCATAAGGGAGAAACTAAAGAGAACCGATACCATTTCTGCCAGGGTGTCT
 CCTGGTTAACGCTGTGGGGAACTGTGCCTGTTCTCCCACAATTGCAATGAATGTCAATGTGCCAAGCAAAGTT
 ACTAAAAAAATACCACGAGGTCC
 >3684109H1 HEAANOT01 INCYTE
 ATTTCATCTCAGGATTACAGTCATTCTGAAAANAGGAGAAATCAAACANAATTAGGAGTTGTGCAACAGCTTTGA
 GAGGAGGCCTAAAGGACAGGAGAAAGGTCTCAATCGTGGAAANAAAATTAAATGTTGATTAAATAGATCACAGCTA
 GTTTCAGAGTACCATGTACGTATTCCACTAGCTGGGTCTGTATTCAGTTCTCGATACGGCTTAGGGTAATGTCAG
 TACAGGAAAAAAACTGTGCAAGTGAGCACCTGATTCCGTGCCTGCT
 >2549720H1 LUNGUT06 INCYTE
 TTAGCTTGGNAAATTCTAAACACAATTGTTATAGCCAGAGGAACAAAGATGATATAAAATATGTTGCTCTGACAAA
 AATACATGATTTCATTCTCGTATGGTGTAGAGTTAGATTAACTGCATTAAAAAAACTGAATTGGAATAGAATTGGT
 AAGTTGCAAAAGACTTTGAAAATAATTAAATTATCATATCTTCATTCTGTATTGGAGATGAAAATAAAAGCAACT
 TATGANAGTAG
 >877279H1 LUNGAST01 INCYTE
 CTTTTTATGACAACTTAGATCAACTATTTTAGCTTGGTAAATTTCTAAACACAATTGTTAGCCAGAGGAACAAA
 GATGATATAAAATATTGTTGCTCTGACAAAAAATACATGATTTCATTCTCGTATGGTGTAGAGTTAGATTAACTGCAT
 TTTAAAAAAACTGAATTGGAATAGAATTGGTAAGTTGCAAAAGGCTTTGAAAATAATTATCATATCTTCATTCC
 TGTTATTGGNGG
 >4713188H1 BRAIHCT01 INCYTE
 CAAAGTTACTAAAAAATACCACGAGGTCTTCAGTTGAGACCAAAGACCGGTGTCAAGGGGATTGCAACAAATCACTCACCG
 ACAGTGGCCCTGGAGCACCATGAGGAGTGTGACTGTGTGCAAGAGGGACACAGGAGGATAGCCGCATCACCAACCAGCAG
 CTCTGCCAGAGCTGTGCACTGGCTGATTCTATTAGAGAACGTTAGCATTCTCCATCCTTAATCTCAGTTGT
 TTGCT
 >2171082H1 ENDNOT03 INCYTE
 AGATAAAACCTGAAAAGAAGAGTGGCCTTATCTCACTTTATCGATAAGTCAGTTATTGTTCATTGTGTACATTTTA
 TATTCTCCTTTGACATTATAACTGTTGGCTTTCTAATCTGTAAATATCTATTACCAAAAGGTATTTAATT
 CTTTTTATGACAACTTAGATCAACTATTTAGCTTGGTAAATTTCTAAACACAATTGTTAGCCAGAGGAACAAA
 GATGA
 >875860H1 LUNGAST01 INCYTE
 CTGGATTTTCATATTCTTATTTAAATTCTGCCATTAGAAGAAGAGAACTACATTCTAGGTTGGAACAGAGATAAAC
 TGAAAAGAAGAGTGGCCTTATCTCACTTTATCGATAAGTCAGTTATTGTTCATTGTGTACATTTATATTCTCCT
 TTGACATTATAACTGTTGGCTTTCTAATCTGTAAATATCTATTACCAAAAGGTATTTAATTCTTTTAT
 GAC
 >706168H1 SYNORAT04 INCYTE
 GCTCATATTACACATATGTAACACAGAACATTCTATGTACTACAAACCTGGTTTTAAAAAGGANCTATGTTGCTATGAAT
 TAAACTGTGTCGTGTGATAGGACAGACTGGATTTCATATTCTTATTTCTGCCATTAGAAGAAGAGAAC
 TACATTCTAGGTTGGAAGAGATAAAACCTGAAAAGAAGAGTGGCCTTATCTCANTTTATCGATAAGTCAGTTATTGTT
 TTCA
 >458823H1 KERANOT01 INCYTE
 ANGAGTTGCCAGAGCTGTGCACTGGCTGATTCTATTAGAGAACGTATGCGTTATCTCCATCCTTAATCTCAGTT
 GTTGNTTCAGGACCTTTCATCTCAGGATTACAGTCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTG
 CAACAGCTTTGAGAGGAGGCCAAAGGNCAAGGAGAAAAGGTCTCAATCGTGGAAAGAAAATTAAATGTTGATTAA
 ATAGATC
 >538436H1 LNODNOT02 INCYTE
 AAAGATGATATAAAATATTGTTGCTCTGACAAAATACATGATTTCATTCTCGTATGGTGTAGAGTTAGATTAACTG
 CATTAAACTGAATTGGAATAGAATTGTAAGTTGCAAAAGACTTTGAAAATAATTAAATTATCATATCTCCAT
 TCCTGTTATTGGAGATGAAAATAAAAGCAACTTATGAAAGTAGACATTCACTGAGCCATTACTAACCTAT
 >1303909H1 PLACNOT02 INCYTE
 AGGAAATCAAATTAGGATAAGATTGTATCTGATGAATATTTCTCTGAACCTCTAACAGAGGAGGTAAAGATTATAC
 AGCTGCACACCTCGTAACCTCTCAGTGTCCATAAGGGAGAAACTAAAGAGAACCGATACCATTCTGCCAGGGTGTCT
 CCTGGTTAACGCTGTGGGGAACTGTGCCTGTTCTCCACAATTGCAATGAATGTCAATGTGCCAAG
 >2739211H1 OVARNOT09 INCYTE
 GTGCATTCTGAAAGAGGAGACATCAAACAGAATTAGGAGTTGTGCAACAGCTTTGAGAGGAGGCCAAAGGACAGGA
 GAAAAGGTCTCAATCGTGGAAAGAAAATTAAATGTTGATTAAATAGATCACCGACTAGTTCAGAGTTACATGTAC
 TATTCCACTAGCTGGGTTCTGTATTCACTGTTGCAACAGGCTTAGGGTAATGTCAAGTACAGGAAAAAAACTGTGCAA
 GTGAGCACCTGAT

FIG. 15 (CONTINUED 2).

>2550343H1 LUNGUT06 INCYTE
TGTACATTTTATATTCTCCTTTGACATTATAACTGTTGGCTTTCNAATCTTGTAAATATATCTATTTTACCAAAG
GTATTTAATATTCTTTTATGACAACCTAGATCAACTATTAGCTGGTAAATTCTAAACACAATTGTTAGC
CAGAGGAACAAAGATGATATAAAATATTGTTGCTCTGACAAAAACATGTATTCATTCTCGTATGGTGC
>5321148H1 FIBPFEN06 INCYTE
CACAAATTGTTAGGCCAGAGGAACAAAGATGATATAAAATATTGTTGCTCTGNAAAAACATGTATTCATTCTCGTA
TGGTCTAGAGTTAGATTAATCTGCATTTAAAAAACTGAATTGGAATAGAATTGTAAGTTGCAAAGACTTTGAAA
TAATTAATTATCATATCTCCATTCTGTTATTGGAGATGAAAATAAAAGCAACTATGAAAGTAAATTAGATCCAC
CATTACTAAC
>879495H1 THYRNOT02 INCYTE
ATTTCATTCTCGTATGGTCTAGAGTTAGATTAATCTGCATTTAAAAAACTGAATTGGAATAGAATTGTAAGTTGCAA
AGACTTTTGAAAATAATTAAATTATCATATCTCCATTCTGTTATTGGAGATGAAAATAAAAGCAACTATGAAAGT
AGACATTCACTGAGATCCAGCCATTACTAACCTATTCTCTTTTGAGGAACTGAGCCTAGCTCAGAAAACATAAGCACC
TGAAAAA
>3325591H1 PTHYNNOT03 INCYTE
TGCAACAGCTTTGAGAGGAGGCCCTAAAGGACAGGAGAAAAGGTCTCAATCGTGGAAAGAAAATTAATGTTGATT
AAATAGATCACCAGCTAGTTCAAGGTACCATGTACGTATCCACTAGCTGGTCTGTATTCAGTTCTTCGATACG
GCTTAGGGTAATGTCAGTACAGGAAAAAACTGTGCAAGTGAGCACCTGATTCCGTTGCCCTGCTAACCTAAAGCNCC
ATGTCNNGGCNAAANCAGAAAAT
>543890H1 OVARNOT02 INCYTE
TTTCTAAACACAATTGTTAGCCAGAGGAACAAAGATGATATAAAATATTGTTGCTCTGACAAAAACATGTATTC
TTCTCGTATGGTCTAGAGTTAGATTAATCTGCATTTAAAAAACTGAATTGGAATAGAATTGTAAGTTGCAAAGNC
TTGAAAATAATTAAATTATCATATCTCCATTCTGTTATTGGAGGATGAAAATAAAAGCAACTATGAAAGTAGG
ACATTCACTGAGATC
>3733565H1 SMCCNOS01 INCYTE
CCTTAATCTCAGTTGTTGCTTCAAGGACCTTCATCTCAGGATTACAGTCATTCTGNAAGANGAGACATCAAACAG
AATTAGGNGTTGCAAAGCTCTTTGAGAGGAGGCCCTAAAGGACAGGAGAAAAGGTCTNCAATCGTGGAAAGNAAATT
AAATGTTGATNAATNGATCACCAGCTAGTTCAAGGTACCATGTACGTATTCACAGCTGGNCNGTATTCACTG
TTCGGAACGGCTTAGGTAATGTCAGTACAGGAAAACAGTGTGAGTGAG
>4641939H1 PROSTMT03 INCYTE
GTAACACACCTGGTTTTAAAAGGAACATGTTGCTATGAATTAAACTTGTGTCATGCTGATAGGACAGACTGGAT
TTTNCAATTTCTTATTAAAATTCTGCCATTAGAAGAAGAGAACTACATTGATGGTTGGNAGAGATAAACCTGAAA
GAAGAGTGGCTTATCTCACTTATCGATAAGTCAGTTATTGTTCATGTCATGTCATTTATATTCTCCTTGACAT
ATAACGTGGCTT
>2007780H1 TESTNOT03 INCYTE
TTATATTCTCCTTTGACATTATAACTGTTGGCTTTCAATCTTGTAAATATATCTATTTTACCAAAGGTATTTAAT
ATTCTTTTTATGACAACCTAGATCAACTATTAGCTGGTAAATTCTAAACACAATTGTTAGCCAGAGGAAC
AAAGATGATATAAAATATTGTTGCTCTGAAAATACATGTAT
>3085331H1 HEAONOT03 INCYTE
GCTCATATTACACATATGTAACCAAGAACATTCTATGTAACACAACTGGTTTTAAAAGGAACATTGCTATGAATT
AAACTTGTGTCGTGCTGATAGGACAGACTGGNTTTTCATATTCTTATTANAATTCTGCCATTAGAAGAAGAGAACTA
CATTCATGGTTGGAAGAGATAAACCTGAAAAGAAGAGTGGCCTATTCACTTATCGATAAGTCAGT
>3414043H1 PTHYNNOT04 INCYTE
GCTCATATTACACATATGTAACCAAGAACATTCTATGTAACACAACTGGTTTTAAAAGGAACATTGTTGCTATGAAT
TAAACTGTCGTGCTGATAGGACAGACTGGATTTCATATTCTTATTAAAATTCTGCCATTAGAAGAAGAGAAC
TACATTCACTGGTTGGAAGAGATAAACCTGAAA
>3705963H1 PENCNOT07 INCYTE
ANACTGTGCAAGTGAGCACCTGATTCCGTTGCTTAACCTAAAGCTCCATGTCCTGGCCTAAATCGTATAAAA
TCTGGAAAC
AAAAGGAACATGTTGCTATGAATTAAACTTGTGTCGTGCTGATAGGACAGACTGGATTTCATATTCTTATTAAAAT
TTCTGCCATTAGAAGAAGAGAACTACNTTCANGGTTGGAAGAGATAACCCCTGAAAAGANGGG
>5137051H1 OVARDIT04 INCYTE
AAAAAAACTGAATTGGAATAGAATTGTAAGTGCAGGAAAGACTNTTGAAAATAATTAAATTATCATATCTCCATTCTGT
TATTGGAGATGAANATAAAAAGCAACTTATGAAAGTAGACATTCACTGAGATCCAGCCATTACTAACCTATTCTTTGGGG
AAATCTGAGCCTAGCTCAGAAAACATAAAGCACCTTGAAAAGACTTGGCAGCTCCTGATAAGCGTGCTGTNTGTCA
GTAGGAACACATCCTATTGATGNTGTTATTAT
>3554223H1 SYNONOT01 INCYTE
ATTAATAGATCACCAGCTAGTTCAAGAGTTACCATGTACGTATTCCACTAGCTGGTTCTGTATTTCACTGTTGAT
ACGGCTTAGGGTAATGTCAGTACAGGAAAAACAGTGCAGCACCTGATTCCGTTGCCCTGCTTAACCTAAAG

FIG. 15 (CONTINUED 3).

CTCCATGTCTGGGCTAAATCGATAAAACTGGATTNTTTTGCATATTACATATGTAAACCAGN
 ACATTCTATGTACNACAAACCTGGTTTAAAAGGAAC
 >4507477H1 OVARTDT01 INCYTE
 GGCTAGTTCAGAGTACCATGTACGTATTCACTAGCTGGGTCGTATTCAGTTCTGATACGGCTTAGGGTAAT
 GTCACTACAGAAAAAACTGTCAAGTGAGCACCTGATTCCGTGCTTAACCTAAAGCTCCATGCTGGGCC
 TAAAATCGTATAAAACTGGGA
 >1955646H1 CONNNOT01 INCYTE
 TGGTAAGTTGCAAAGACTTTGAAAATAATTAAATTATCATATCTTCCATTCTGTTATTGGAGATGAAAATAAAAGC
 AACTTATGAAAGTAGACATTCACTACAGCTCCAGCATTACTAACCTATTCTTTGGGAAATCTGAGCCTAGCTCAGAAAA
 ACATAAAGCCTTGAAAAGACTTGGCAGCTTCGTGATAAAAGCGTGTGCTGTGAGTAGGAAACACATCCTATT
 TTGTGATGTTGGTTTATATCCTAAACC
 >4163378H1 BRSTNOT32 INCYTE
 AATAGATCACCAGCTAGTTTCAAGTACGTACGTTACCATGTACGTATTCCACTAGCTGGNTCTGTATTCAGTTCTTCGATACG
 GCTTAGGGTAATGTCACTACAGGAAAAAAAGCTGTGCAAGTGAGCACCTGATTCCGTGCTGCTTAACCTAAAGCTCC
 ATGTCCTGGGCCAAAATCGTATA
 >5095141H1 EPIMNON05 INCYTE
 AGATAAACCTGAAAAGAAGAGTGGCTTATNTTCACTTATCGATAAGTCAGNTTATTGTTTCAATTGTGTCACATTNN
 TATTCTCCTTTGACATTATAACTGNTGGCTTCTAACNTGTTAAATATCTATTACCAAGGTATTAAATATT
 CTTT
 >943826H1 ADRENOT03 INCYTE
 TATGGTCTAGAGTTAGATTATCTGCATTAAAATGAAATTGGAATAGAATTGTAAGTTGCAAAGACTTTGAA
 AATAATTAAATTATCATATCTTCCATTCTGTTATTGGAGATGAAAATAAAAGCAACTTATG
 >3451273H1 UTRSNON03 INCYTE
 TTTTTNTTTGCTCATATTACATATGTAACCNGAACATTCTATGTACNACAAACCTGGTTTAAAAGGAACATAG
 TTGCTATGAATTAAACTGTGTCGTGCTGATAGGACAGACTGGATTTCANATTCTTANTAAANNNTCTGCCATTAG
 AAGA
 >1402278H1 LATRTUT02 INCYTE
 GTACAGGAAAAAAACTGTGCAAGTGAGCACCTGATTCCGTGCTTAACTCTAAAGCTCCATGCTCTGGGCCAAA
 ATCGTATAAAATCTGGAnnnnnnnnnnnnnnnnnnGCTCATATTACATATGTAACCAACATTCTATGTACTACAAA
 CCTGGTTTAAAAGGAACATATGTTCTATGAATTAAACTTGTGTCGTGCTGATAGGACAGACTGGATTTCATATT
 CTTA
 >4361191H1 SKIRNOT01 INCYTE
 GCAAAGACTTTGANAATNATTAAATTATCATATCTTCCATTCTGTTATNGGAGATGANAATAAAAGCAACTTATGA
 AAGTAGACATTCACTACAGCCATTACTAACCTATTCTTTGGGAAATCTGAGCCTAGCNCAGAAAACATAAAGC
 ACCTTGAAAAGACTTGGCAGCTCCTGATAAAAGCGTGTGCTGTGAGTAGGAACACATCCNATTATTGTGNTG
 GNNGTTTATGATC
 >1307017H1 PLACNOT02 INCYTE
 TGTCAGTACAGGAAAAAAACTGTGCAAGTGAGCACCTGATTCCGTGCTTGTAACTCTAAAGCTCCATGCTCTGGC
 CTAAAATCGTATAAAATCTGGAnnnnnnnnnnnnnnnnGCTCATATTACATATGTAACCAACATTCTATGTACT
 ACAAAACCTGGTTTAAAAGGAACATATGTTCTATGAATTAAACTTGTGTCATGCTGATAGGACAGACTGGATTTC
 TAT
 >5032225H1 HEARFET03 INCYTE
 AATTATCATATCTTCCATTCTGTTATTGGAGATGNAATAAAAGCAACTTATGAAAGTAGACATTCACTACAGCCAT
 TACTAACCTATTCTTTGGGAAATCTGAGCCTAGCTCAGAAAACATAAAGCACCTTGAAAAGACTGTCAGCTTC
 CTGATAAAAGCGTGTGCTGTGCACTAGGAACACATCTTATTGTGATGTTGGTTTATTATCTAAACTCTGTT
 CCAT
 >3732621H1 SMCCNOS01 INCYTE
 ANAGATGATATAAAANATTGTTGCTCTGACAANNATACATGTATTCTCATGCTATGGTGCTAGAGTTAGATTATCTG
 CNTTTAAAAGACTGANITGGAATAGANTTGGTAAGTTGCAAAGNCNTTGAAAATNATTAAAGTTATCAGAT
 >3530274H1 BLADNOT09 INCYTE
 TTCCATTCTGTTATTGGAGATGAAAATAAAAGCAACTTATGAAAGTAGACATTCACTACAGCCATTACTAACCTATT
 CCTTTTTGGGAAATCTGAGCCTAGCTCAGAAAACATAAAGCACCTTGAAAAGACTGGCAGCTCCTGATAAAGCG
 TGCTGTGCTGTGCACTAGGAACACATCTTATTGTGATGTTGGTTTATTATCTAAACTCTGTTCCATACACTTG
 TATAAATACATGGATATTGTACAGAAGTATGTCTTAAACCAGTTCA
 >3530249H1 BLADNOT09 INCYTE
 CTTCCATTCTGTTATTGGAGATGAAAATAAAAGCAACTTATGAAAGTAGACATTCACTACAGCCATTACTAACCTATT
 TCCTTTTTGGGAAATCTGAGCCTAGCTCAGAAAACATAAAGCACCTTGAAAAGACTGGCAGCTCCTGATAAAGC
 GTGCTGTGCTGTGCACTAGGAACACATCTTATTGTGATGTTGGTTTATTATCTAAACTCTGTTCCATACACT
 TGATAAAATACATGGATATTGTACAGAAGTATGTCTTAAACCAGTTCACTTATGTACCTGG

FIG. 16.

VEGFE1	AAAATGTATGGATAACAACCTAC	22
VEGFE2	GTTGATGAAAGATTGGGCTTG	23
VEGFE3	TTTCTAAAGGAAATCAAATTAG	22
VEGFE4	GATAAGATTGTATCTGATG	20
VEGFE5	GATGTCTCCTCTTCAG	17
VEGFE6	GCACAACTCCTAATTCTG	18
VEGFE7	AGCACCTGATTCCGTG	19
VEGFE8	TAGTACATAGAATGTTCTGG	20
VEGFE9	AAGAGACATACTCTGTAC	19
VEGFE10	CCAGGTACAATAAGTGAAGT	21

FIG. 17.

+3
N L L T E E V R L Y M N I F L L

] -----

1 AGGAAATCAA ATTAGGATAA GATTTGTATC TGATGAATAT TTTCCTTCTG
AACCTTCTAA CAGAGGAGGT AAGATTATAC
TCCTTTAGTT TAATCCTATT CTAAACATAG ACTACTTATA AAAGGAAGAC
TTGGAAGATT GTCTCCTCCA TTCTAATATG

+3 S C T P R N F S V S I R E E L K R
T D T I F W P G C L
-----]

81 AGCTGCACAC CTCGTAACCT CTCAGTGTCC ATAAGGAAAG AACTAAAGAG
AACCGATACC ATTTTCTGGC CAGGTTGTCT
TCGACGTGTG GAGCATTGAA GAGTCACAGG TATTCCCTTC TTGATTTCTC
TTGGCTATGG TAAAAGACCG GTCCAACAGA

-2

+3 L V K R C G G N C A C C L H N C N
E C Q C V P S K V

161 CCTGGTTAAA CGCTGTGGTG GGAACTGTGC CTGTTGTCTC CACAATTGCA
ATGAATGTCA ATGTGTCCCA AGCAAAGTTA
GGACCAATTG GCGACACAC CCTTGACACG GACAACAGAG GTGTTAACGT
TACTTACAGT TACACAGGGT TCGTTCAAT

-2

+3 T K K Y H E V L Q L R P K T G V R
G L H K S L T D V A

+1
D C T N H S P T W P V S G
-----]

241 CTAAAAAATA CCACGAGGTC CTTCAGTTGA GACCAAAGAC CGGTGTCAGG
GGATTGCACA AATCACTCAC CGACGTGGCC
GATTTTTAT GGTGCTCCAG GAAGTCAACT CTGGTTCTG GCCACAGTCC
CCTAACGTGT TTAGTGAGTG GCTGCACCGG

-2
-----[

+3 L E H H E E C D C V C R G S T G G

FIG. 17 (CONTINUED).

> +2 I A A S P P A A L A V Q R E H R R
 -----]
 ----- +1 W S T M R S V T V C A E G A Q E D
 S R I T T S S S C

 321 CTGGAGCACC ATGAGGAGTG TGACTGTGTG TGCAGAGGGA GCACAGGAGG
 ATAGCCGCAT CACCACCAGC AGCTCTTGCC
 GACCTCGTGG TACTCCTCAC ACTGACACAC ACGTCTCCCT CGTGTCCCTCC
 TATCGGGCGTA GTGGTGCTCG TCGAGAACGG
 ----- +2 Q S C A V Q W L I L L E N V C V I
 S I L N L S C L L Q
 ----- +1 P E L C S A V A D S I R E R M R Y
 L H P
 ----->
 401 CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT
 CTCCATCCTT AATCTCAGTT GTTGCTTC
 GTCTCGACAC GTCACGTCAC CGACTAAGAT AATCTCTTGC ATACGCAATA
 GAGGTAGGAA TTAGAGTCAA CAAACGAAGT
 ----- +2 G P F I F R I Y S A F
 ----->
 481 AGGACCTTTC ATCTTCAGGA TTTACAGTG ATTCTGAAAG AGGAGACATC
 AAACAGAATT AGGAGTTGTG CAACAGCTCT
 TCCTGGAAAG TAGAAGTCCT AAATGTCACG TAAGACTTTTC TCCTCTGTAG
 TTTGTCTTAA CCCTCAACAC GTTGTGAGA
 ----- 561 TTTGAGAGGA GGCCTAAAGG ACAGGAGAAA AGGTCTCAA TCGTGGAAAG
 AAAATTAAAT GTTGTATTAA ATAGATCACC
 AAACCTCTCCT CC GGATTTC TGTCTCTTT TCCAGAAGTT AGCACCTTTC
 TTTTAATTAA CAACATAATT TATCTAGTGG
 ----- 641 AGCTAGTTTC AGAGTTACCA TGTACGTATT CCACTAGCTG GGTTCTGTAT
 TTCAGTTCTT TCGATACGGC TTAGGGTAAT
 TCGATCAAAG TCTCAATGGT ACATGCATAA GGTGATCGAC CCAAGACATA
 AAGTCAAGAA AGCTATGCCG AATCCCATTAA
 ----- 721 GTCAGTACAG GAAAAAAACT GTGCAAGTGA GCACCTGATT CCGTTGCCTT
 GGCTTAACTC TAAAGCTCCA TGTCTGGGC
 CAGTCATGTC CTTTTTTGGA CACGTTCACT CGTGGACTAA GGCAACGGAA
 CCGAATTGAG ATTTGAGGT ACAGGACCCG
 ----- 801 CTAAAATCGT ATAAAATCTG GA
 GATTTTAGCA TATTTAGAC CT

FIG. 18.

+3
N L L T E E V R L Y M N I F L L
-----]

1 AGGAAATCAA ATTAGGATAA GATTGTATC TGATGAATAT TTTCTTCTG
AACCTTCTAA CAGAGGAGGT AAGATTATAC
TCCTTAGTT TAATCCTATT CTAAACATAG ACTACTTATA AAAGGAAGAC
TTGGAAGATT GTCTCCTCCA TTCTAATATG

+3 S C T P R N F S V S I R E E L K R
T D T I F W P G C L -----]

81 AGCTGCACAC CTCGTAACCT CTCAGTGTCC ATAAGGGAAG AACTAAAGAG
AACCGATACC ATTTCTGGC CAGGTTGTCT
TCGACGTGTG GAGCATTGAA GAGTCACAGG TATTCCCTTC TTGATTTCTC
TTGGCTATGG TAAAAGACCG GTCCAACAGA
-2 -----<-----

+3 L V K R C G G N C A C C L H N C N
E C Q C V P S K V -----]

161 CCTGGTTAAA CGCTGTGGTG GGAACTGTGC CTGTTGTCTC CACAATTGCA
ATGAATGTCA ATGTGTCCA AGCAAAGTTA
GGACCAATTG GCGACACCAC CCTTGACACG GACAACAGAG GTGTTAACGT
TACTTACAGT TACACAGGGT TCGTTTCAAT
-2 -----]

+3 T K K Y H E V L Q L R P K T G V R
G L H K S L T D V A -----]

+1
D C T N H S P T W P V S G
-----]

241 CTAACGGAGGTC CTTCAGTTGA GACCAAAGAC CGGTGTCAGG
GGATTGCACA AATCACTCAC CGACGTGGCC
GATTTTTTAT GGTGCTCCAG GAAGTCAACT CTGGTTCTG GCCACAGTCC
CCTAACGTGT TTAGTGAGTG GCTGCACCGG

FIG. 18 (CONTINUED 1).

-2 -----[
+3 L E H H E E C D C V C R G S T G G
>
+2
I A A S P P A A L A V Q R E H R R
]

+1 W S T M R S V T V C A E G A Q E D
S R I T T S S S C

321 CTGGAGCACC ATGAGGAGTG TGACTGTGTG TGCAGAGGGA GCACAGGAGG
ATAGCCGCAT CACCACCAGC AGCTCTGCC
GACCTCGTGG TACTCCTCAC ACTGACACAC ACGTCTCCCT CGTGTCCCTCC
TATCGGCGTA GTGGTGGTCG TCGAGAACGG

+2 Q S C A V Q W L I L L E N V C V I
S I L N L S C L L Q

+1 P E L C S A V A D S I R E R M R Y
L H P
----->
401 CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT
CTCCATCCTT AATCTCAGTT GTTGCGTTCA
GTCTCGACAC GTCACGTAC ACAGTAAGAT AATCTCTTGC ATACGCAATA
GAGGTAGGAA TTAGAGTCAA CAAACGAAGT

+2 G P F I F R I Y S A F
----->
481 AGGACCTTTC ATCTTCAGGA TTTACAGTGC ATTCTGAAAG AGGAGACATC
AAACAGAATT AGGAGTTGTG CAACAGCTCT
TCCTGGAAAG TAGAAGTCCT AAATGTCACG TAAGACTTTC TCCTCTGTAG
TTTGTCTTAA TCCTCAACAC GTTGTGAGA

561 TTTGAGAGGA GGCCTAAAGG ACAGGAGAAA AGGTCTCAA TCGTGGAAAG
AAAATTAAT GTTGTATTAA ATAGATCACC
AAACTCTCCT CC GGATTTC TGT CCTCTTT TCCAGAAGTT AGCACCTTTC
TTTAATTAA CAACATAATT TATCTAGTGG

641 AGCTAGTTTC AGAGTTACCA TGTACGTATT CCACTAGCTG GGTTCTGTAT
TTCAGTTCTT TCGATACGGC TTAGGGTAAT
TCGATCAAAG TCTCAATGGT ACATGCATAA GGTGATCGAC CCAAGACATA
AAGTCAAGAA AGCTATGCCG AATCCCATTAA

721 GTCAGTACAG GAAAAAAACT GTGCAAGTGA GCACCTGATT CCGTTGCCCTT
GGCTTAACTC TAAAGCTCCA TGTCCCTGGC

FIG. 18 (CONTINUED 2).

CAGTCATGTC CTTTTTTGGA CACGTTCACT CGTGGACTAA GGCAACGGAA
CCGAATTGAG ATTTCGAGGT ACAGGACCCG

801 CTAAAATCGT ATAAAATCTG GATTTTTTN TTTTTTTTG CGCATATTCA
CATATGTAAA CCAGAACATT CTATGTACTA
GATTTTAGCA TATTTTAGAC CTAAAAAAAN AAAAAAAAAC GCGTATAAGT
GTATACATTT GGTCTTGAA GATACATGAT

881 CAAACCTGGT TTTAAAAAG GAACTATGTT GCTATGAATT AAACTTGTGT
CGTGCTGATA GGACAGACTG GATTTTCAT
GTTTGGACCA AAAATTTTC CTTGATACAA CGATACTTAA TTTGAACACA
GCACGACTAT CCTGTCTGAC CTAAAAAGTA

-3

<-----

961 ATTTCTTATT AAAATTTCTG CCATTTAGAA GAAGAGAACT ACATTGATGG
TTTGGAAAGAG ATAACACCTGA AAAGAAAGAGT
TAAAGAATAA TTTAAAGAC GGTAAATCTT CTTCTCTTGA TGTAAGTACC
AACCTTCTC TATTTGGACT TTTCTTCTCA

-3

1041 GGCCTTATCT TCACCTTATC GATAAGTCAG TTTATTTGTT TCATTGTGTA
CATTTTTATA TTCTCCTTT GACATTATAA
CCGGAATAGA AGTGAATAG CTATTCAGTC AAATAAACAA AGTAACACAT
GTAAAAATAT AAGAGGAAAA CTGTAATATT

-3

-----[

1121 CTGTTGGCTT TTCTAATCTT GTTAAATATA TCTATTTTA CCAAAGGTAT
TTAATATTCT TTTTTATGAC AACTTAGATC
GACAACCGAA AAGATTAGAA CAATTTATAT AGATAAAAAT GGTTTCCATA
AATTATAAGA AAAAATACTG TTGAATCTAG

1201 AACTATTTT AGCTTGGTAA ATTTTCTAA ACACAATTGT TATAGCCAGA
GGAACAAAGA TGATATAAAA TATTGTTGCT
TTGATAAAAAA TCGAACCAATT TAAAAAGATT TGTGTTAACAA ATATCGGTCT
CCTTGTCT ACTATATTT ATAACAACGA

1281 CTGACAAAAA TACATGTATT TCATTCTCGT ATGGTGCTAG AGTTAGATTA
ATCTGCATTT TAAAAAACTG AATTGGAATA
GACTGTTTT ATGTACATAA AGTAAGAGCA TACCACGATC TCAATCTAAT
TAGACGTAAA ATTTTTGAC TTAACCTTAT

1361 GAATTGGTAA GTGCAAAGA CTTTTGAAA ATAATTAAAT TATCATATCT
TCCATTCCGT TTATTGGAGA TGAAAATAAA
CTTAACCATT CAACGTTCT GAAAAACTTT TATTAATTAA ATAGTATAGA
AGGTAAGGAC AATAACCTCT ACTTTTATTT

1441 AAGCAACTTA TGAAAGTAGA CATTCACTG CAGCCATTAC TAACCTATTG
CTTTTTGGG GAAATCTGAG CCTAGCTCAG
TTCGTTGAAT ACTTTCTACT GTAAAGTCTAG GTCGGTAATG ATTGGATAAG
GAAAAAAACCC CTTTAGACTC GGATCGAGTC

FIG. 18 (CONTINUED 3).

1521 AAAAACATAA AGCACCTTGA AAAAGACTTG GCAGCTTCCT GATAAAGCGT
GCTGTGCTGT GCAGTAGGAA CACATCCTAT
TTTTTGTATT TCGTGGAACT TTTTCTGAAC CGTCGAAGGA CTATTCGCA
CGACACGACA CGTCATCCTT GTGTAGGATA

1601 TTATTGTGAT GTTGTGGTTT TATTATCTTA AACTCTGTT CATAACATTG
TATAAATACA TGGATATTTT TATGTACAGA
AATAACACTA CAACACCAAA ATAATAGAAT TTGAGACAAG GTATGTGAAC
ATATTTATGT ACCTATAAAA ATACATGTCT

1681 AGTATGTCTC TTAACCAGTT CACTTATTGT ACCTGG
TCATACAGAG AATTGGTCAA GTGAATAACA TGGACC

FIG. 19. DNA and polypeptide sequence used for mammalian cell expression

+1 m s l f g 1 1 1 l t s a l a g q r
 1 GGATCCAAAA TGAGCCTCTT CGGGCTTCTC CTGCTGACAT CTGCCCTGGC CGGCCAGAGA

+1 q g t q a E S N L S S K F Q F S S N K E
 61 CAGGGGACTC AGGGGAAATC CAACCTGAGT AGTAAATTCC AGTTTCCAG CAACAAGGAA

+1 Q N G V Q D P Q H E R I I T V S T N G S
 121 CAGAACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA CTGTGTCTAC TAATGGAAGT

+1 I H S P R F P H T Y P R N T V L V W R L
 181 ATTACACAGCC CAAGGTTCC TCATACTTAT CCAAGAAATA CGGTCTTGGT ATGGAGATTA

+1 V A V E E N V W I Q L T F D E R F G L E
 241 GTAGCAGTAG AGGAAAATGT ATGGATACAA CTTACGTTTG ATGAAGATT TGGGCTTGAA

+1 D P E D D I C K Y D F V E V E E P S D G
 301 GACCCAGAAG ATGACATATG CAAGTATGAT TTGAGGAAAG TTGAGGAACC CAGTGATGGA

+1 T I L G R W C G S G T V P G K Q I S K G
 361 ACTATATTAG GGCCTGGTG TGGTTCTGGT ACTGTACAG GAAAACAGAT TTCTAAAGGA

+1 N Q I R I R F V S D E Y F P S E P G F C
 421 AATCAAATTA GGCTAAGATT TGTATCTGAT GAATATTTTC CTTCTGAACC AGGGTTCTGC

+1 I H Y N I V M P Q F T E A V S P S V L P
 481 ATCCACTACA ACATGTGTCAT GCCACAAATC ACAGAAGCTG TGAGCTCTTC AGTGCTACCC

+1 P S A L P L D L L N N A I T A F S T L E
 541 CCTTCAGCTT TGCCACTGGA CCTGCTTAAT AATGCTATAA CTGCCCTTAG TACCTTGGAA

+1 D L I R Y L E P E R W Q L D I E D L Y R
 601 GACCTTATTC GATATCTTGA ACCAGAGAGA TGGCAGTTGG ACTTGAAGA TCTATATAGG

+1 P T W Q L L G K A F V F G R K S R V V D
 661 CCAACTTGGC AACTCTTGG CAAGGCTTTT GTTTTGAA GAAAATCCAG AGTGGTGGAT

+1 L N L L T E E V R L Y S C T P R N F S V
 721 CTGAACCTTC TAACAGAGGA GGTAAGATT A TACAGCTGCA CACCTCGTAA CTTCTCAGTG

+1 S I R E E L K R T D T I F W P G C L L V
 781 TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTCT GGCCAGGTTG TCTCCTGGTT

+1 K R C G G N C A C C L H N C N E C Q C V
 841 AAACGCTGTG GTGCGAACTG TGCCCTGTGT CTCCACAAATT GCAATGAATG TCAATGTGTC

+1 P S K V T K K Y H E V L Q L R P K T G V
 901 CCAAGCAAAG TTACTAAAAA ATACCACGAG GTCCCTCAGT TGAGACCAAA GACCGGTGTC

+1 R G L H K S L T D V A L E H H E E C D C
 961 AGGGGATTGC ACATTTACT CACCGACGTG GCCCTGGAGC ACCATGAGGA GTGTGACTGT

+1 V C R G S T G G S R G P F E G K P I P N
 1021 GTGTGCGAGAG GGACACAGG AGGATCTAGA GGGCCCTTGG AAGGTAAGCC TATCCCTAAC

+1 P L L G L D S T R T G H H H H H H H
 1081 CCTCTCCTCG GTCTCGATTC TACCGCTTACG GGTCTGATTC ACCATCACCA TTGA

FIG. 20. DNA and polypeptide sequence used for baculovirus/insect cell expression

1 GAATTCAAAG GCCTGTATTT TACTGTTTTC GTAACAGTTT TGTAATAAAA AAACCTATAA
+3 m k f l v n v a l v f m v v y i s y i
61 ATATGAAATT CTTAGTCAAC GTTGCCCTTG TTTTATGGT CGTATACATT TCTTACATCT
+3 y a D P E S H H H H H E S N L S S K F
121 ATGCGGATCC GGAGTCTCAC CATCACCAAC ATCATGAATC CAACCTGAGT AGTAAATTCC
+3 Q F S S N K E Q N G V Q D P Q H E R I I
181 AGTTTCCAG CAACAAGGAA CAGAACGGAG TACAAGATCC TCAGCATGAG AGAATTATTA
+3 T V S T N G S I H S P R F P H T Y P R N
241 CTGTGTCTAC TAATGGAAGT ATTACACAGCC CAAGGTTCC TCATACATTAT CCAAGAAATA
+3 T V L V W R L V A V E E N V W I Q L T F
301 CGGTCTTGGT ATGGAGATTA GTAGCAGTAG AGGAAATGT ATGGATACAA CTTACGTTG
+3 D E R F G L E D P E D D I C K Y D F V E
361 ATGAAAGATT TGGCCTTGAA GACCCAGAAG ATGACATATG CAAGTATGAT TTTGTAGAAG
+3 V E E P S D G T I L G R W C G S G T V P
421 TTGAGGAACC CAGTGATGGA ACTATATTAG GGCGCTGGT TGTTCTGGT ACTGTACCAAG
+3 G K Q I S K G N Q I R I R F V S D E Y F
481 GAAAACAGAT TTCTAAAGGA AATCAAATTA GGATAAGATT TGTATCTGAT GAATATTTTC
+3 P S E P G F C I H Y N I V M P Q F T E A
541 CTTCTGAACC AGGGTTCTGC ATCCACTACA ACATTGTCAI GCCACAATTACAGAAGCTG
+3 V S P S V L P P S A L P L D L L N N A I
601 TGAGTCCTTC AGTGCTACCC CCTTCAGCTT TGCCACTGGA CCTGCTTAAT AATGCTATAA
+3 T A F S T L E D L I R Y L E P E R W Q L
661 CTGCCCTTAG TACCTTGAA GACCTTATTC GATATCTGAT ACCAGAGAGA TGGCAGTTGG
+3 D L E D L Y R P T W Q L L G K A F V F G
721 ACTTACAAGA TCTATATAGG CCAACTTGCG AACTTCTGG CAAGGCTTTT GTTTTGAA
+3 R K S R V V D L N L L T E E V R L Y S C
781 GAAAATCCAG AGTGGTGGAT CTGAACCTTC TAACAGAGGA GGTAAGATTA TACAGCTGCA
+3 T P R N F S V S I R E E L K R T D T I F
841 CACCTCGTAA CTTCTCAGTG TCCATAAGGG AAGAACTAAA GAGAACCGAT ACCATTTCT
+3 W P G C L L V K R C G G N C A C C L H N
901 GGCCAGGTTG TCTCTGGTT AACCGCTGTG GTGGGAACGT TGCCCTGTTGT CTCCACAATT
+3 C N E C Q C V P S K V T K K Y H E V L Q
961 GCAATGAATG TCAATGTGTC CCAAGCAAAG TTACTAAAAA ATACCAACGAG GTCCCTTCAGT
+3 L R P K T G V R G L H K S L T D V A L E
1021 TGAGACCAAA GACCGGTGTC AGGGGATTGC ACAAAATCACT CACCGACGTG GCCCTGGAGC
+3 H H E E S D C V C R G S T G G
1081 ACCATGAGGA GTGTGACTGT GTGTGCAAG GGAGCACAGG AGGATAGCTC TAGA

35 / 54

*FIG. 21. DNA and polypeptide sequence used for *E.coli* expression*

+3 O T N S S S N N N N N N N N N N L G I
 1 CGCAGACTAA TTCGAGCTCG AACACAACA ACAATAACAA TAACAACAAC CTCGGGATCG-

+3 E G R I S E F E S N L S S K F Q F S S N
 61 AGGGAGGAT TTCAAGATTG GAATCCAACC TGAGTAGTAA ATTCCAGTTT TCCAGCAACA

+3 K E Q N G V Q D P Q H E R I I T V S T N
 121 AGGAACAGAA CGGAGTACAA GATCCTCAGC ATGAGAGAAT TATTACTGTG TCTACTAATG

+3 G S I H S P R F P H T Y P R N T V L V W
 181 GAAGTATTCA CAGCCCAAGG TTTCCTCATA CTTATCCAAG AAATACGGTC TTGGTATGGA

+3 R L V A V E E N V W I Q L T F D E R F G
 241 GATTAGTAGC AGTAGAGGAA AATGTATGGA TACAACCTAC GTTGATGAA AGATTTGGC

+3 L E D P E D D I C K Y D F V E V E E P S
 301 TTGAAGACCC AGAAGATGAC ATATGCAAGT ATGATTTGT AGAAGTTGAG GAACCCAGTG

+3 D G T I L G R W C G S G T V P G K Q I S
 361 ATGGAACATAT ATTAGGGCGC TGGTGTGGTT CTGGTACTGT ACCAGGAAAA CAGATTCTA

+3 K G N Q I R I R F V S D E Y F P S E P G
 421 AAGGAAATCA ATTAGGATA AGATTTGTAT CTGATGAATA TTTTCCTTCT GAACCAGGGT

+3 F C I H Y N I V M P Q F T E A V S P S V
 481 TCTGCATCCA CTACAACATT GTCATGCCAC AATTACAGA AGCTGTGAGT CCTTCAGTGC

+3 L P P S A L P L D L L N N A I T A F S T
 541 TACCCCCCTTC AGCTTGCCA CTGGACCTGC TTAATAATGC TATAACTGCC TTTAGTACCT

+3 L E D L I R Y L E P E R W Q L D L E D L
 601 TGGAAGACCT TATTCGATAT CTTGAACCAAG AGAGATGGCA GTTGGACTTA GAAGATCTAT

+3 Y R P T W Q L L G K A F V F G R K S R V
 661 ATAGGCCAAC TTGGCAACCT CTTGGCAAGG CTTTTGTTT TGGAAGAAAA TCCAGAGTGG

+3 V D L N L L T E E V R L Y S C T P R N F
 721 TGGATCTGAA CCTTCTAACCA GAGGAGGAA GATTACAG CTGCACACCT CGTAACCTCT

+3 S V S I R E E L K R T D T I F W P G C L
 781 CAGTGTCCAT AAGGAAGAAA CTAAAGAGAA CCGATACCAT TTTCTGGCCA GGTTGTCCTCC

+3 L V K R C G G N C A C C L H N C N E C Q
 841 TGGTTAACG CTGTGGTGGG AACTGTGCCCT GTTGTCTCCA CAATTGCAAT GAATGTCAT

+3 C V P S K V T K K Y H E V L Q L R P K T
 901 GTGTCCAAG CAAAGTTACT AAAAATACC ACGAGGTCCCT TCAGTTGAGA CCAAAGACCG

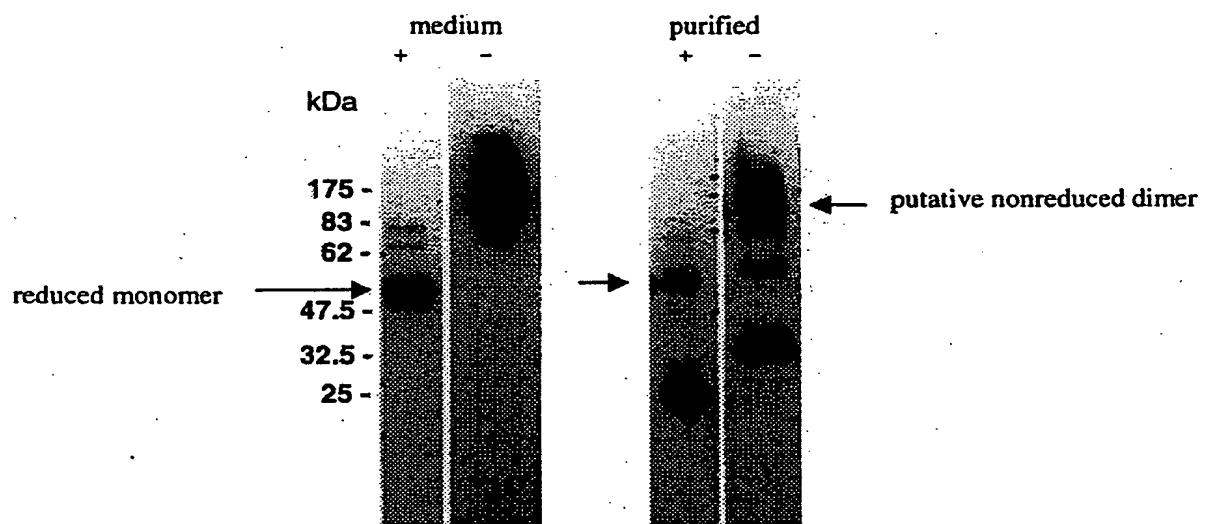
+3 G V R G L H K S L T D V A L E H H E E C
 961 GTGTCAAGGGG ATTGCACAAA TCACTCACCG ACAGTGGCCCT GGAGCACCAC GAGGAGTGTG

+3 D C V C R G S T G G H H H H H H *
 1021 ACTGTGTGTG CAGAGGGAGC ACAGGAGGAC ATCATCACCA TCACCATTGA TCTAGAGTCG

1081 ACCTGCAGGC AAGCTT

FIG. 22. Disulphide-linked dimerisation of VEGF-X

(A) Mammalian cell expression



(B) *E.coli* expression

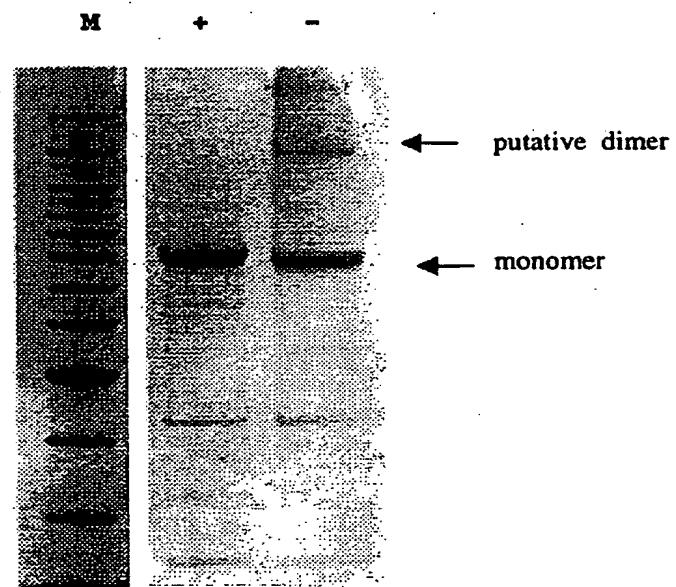


FIG. 23. Glycosylation of VEGF-X

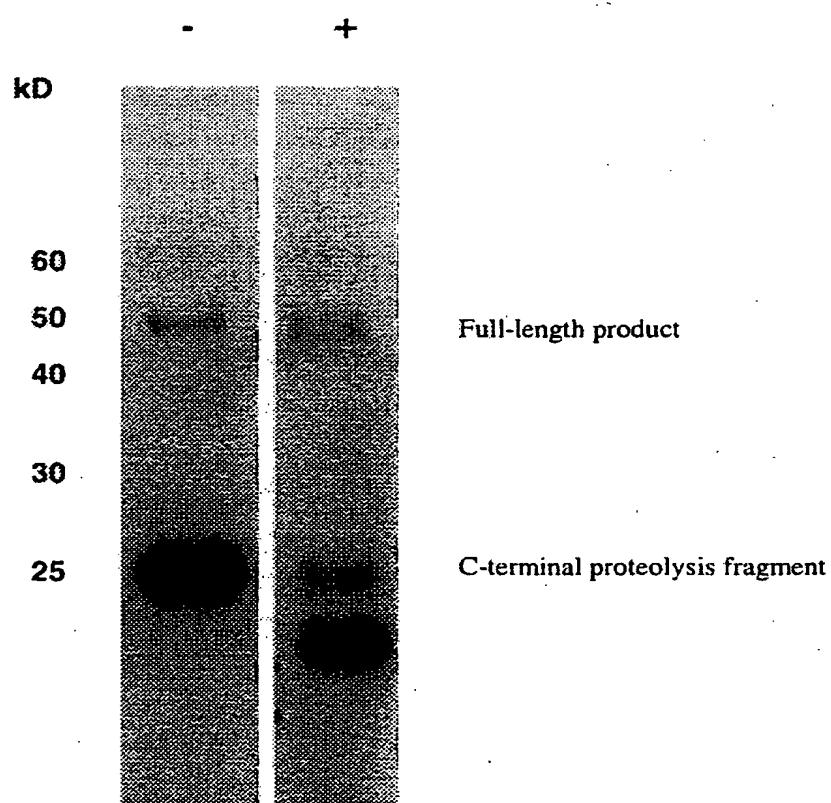


FIG. 24.
DNA and polypeptide sequence used for *E.coli* expression of the PDGF-like domain

+3 M R G S H H H H H H G M A S M
1 AAGGAGATAT ACATATGC GGTTCTCATC ATCATCATCA TCATGGTATG CCTAGCATGA

+3 T G G Q Q M G R D L Y D D D D K D P G R
61 CTGGTGGACA GCATAATGGGT CGGGATCTGT ACGACGATGA CGATAAGGAT CCGGGAAAGAA

+3 K S R V V D L N L L T E E V R L Y S C T
121 AATCCAGAGT GGTGGATCTG AACCTTCTAA CAGAGGAGGT AAGATTATAC AGCTGCACAC

+3 P R N F S V S I R E E L K R T D T I F W
181 CTCGTAACCTT CTCAGTGTCC ATAAGGGAAG AACTAAAGAG AACCGATACC ATTTTCTGGC

+3 P G C L L V K R C G G N C A C C L H N C
241 CAGGGTTCT CCTGGTTAAA CGCTGTGGTG GGAACTGTGC CTGTTGTCTC CACPAATTGCA

+3 N E C Q C V P S K V T K K Y H E V L Q L
301 ATGAATGTCA ATGTGTCCC AGCAAAGTTA CTAAAAAATA CCACGGAGGTC CTTCAGTTGA

+3 R P K T G V R G L H K S L T D V A L E H
361 GACCAAAGAC CGGTGTCAAGG GGATTGCACA AATCACTCAC CGACGTGGCC CTGGAGCACC

+3 H E E C D C V C R G S T G G
421 ATGAGGAGTG TGACTGTGTG TGCAGAGGG ACGACAGGAGG ATAATGAATT CGAAGCTTGA

481 TCCGGCTGCT AACAAAGCCC

*FIG. 25. Expression of PDGF domain in *E.coli**

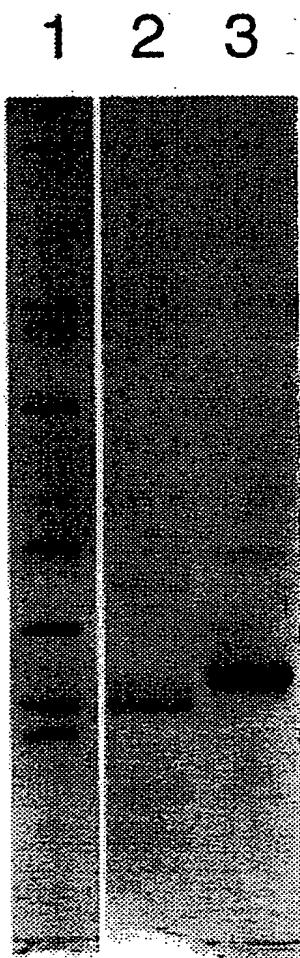


FIG. 26.

DNA and polypeptide sequence used for *E.coli* expression of the CUB-like domain

+2 M A M D I G I N S D P E S H H H H H H H
1 GGCGATGCC ATGGATATCG GAATTAATTG GGATCCGGAG TCTCACCATC ACCACCATCA

+2 E S N L S S K F Q F S S N K E Q N G V Q
61 TGAATCCAAC CTGAGTAGTA AATTCCAGTT TTCCAGAAC AAGGAACAGA ACGGAGTACA

+2 D P Q H E R I I T V S T N G S I H S P R
121 AGATCCTCAG CATGAGAGAA TTATTACTGT GTCTACTAAT GGAAGTATTG ACAGCCCAAG

+2 F P H T Y P R N T V L V W R L V A V E E
181 GTTCCTCAT ACTTATCCAA GAAATACGGT CTTGGTATGG AGATTAGTAG CAGTAGAGGA

+2 N V W I Q L T F D E R F G L E D P E D D
241 AAATGTATGG ATACAACCTTA CGTTTGATGA AAGATTGGG CTTGAAGACC CAGAAGATGA

+2 I C K Y D F V E V E E P S D G T I L G R
301 CATATGCAAG TATGATTTG TAGAAGTTGA GGAACCCAGT GATGGAACTA TATTAGGGCG

+2 W C G S G T V P G K Q I S K G N Q I R I
361 CTGGTGTGGT TCTGGTACTG TACCAAGAAA ACAGATTCT AAAGGAAATC AAATTAGGAT

+2 R F V S D E Y F P S E P G F C I H Y N I
421 AAGATTGTA TCTGATGAAT ATTTCCCTTC TGAACCAGGG TTCTGCATCC ACTACAACAT

+2 V M P C F T E A V
481 TGTCATGCCA CAATTACAG AAGCTGTGTA GTCGAGCTCC GTCGACAAGC TTGCGGCCGC

541 ACTCGAGCAC

41 / 54

FIG. 27. Expression of the CUB domain in *E.coli*

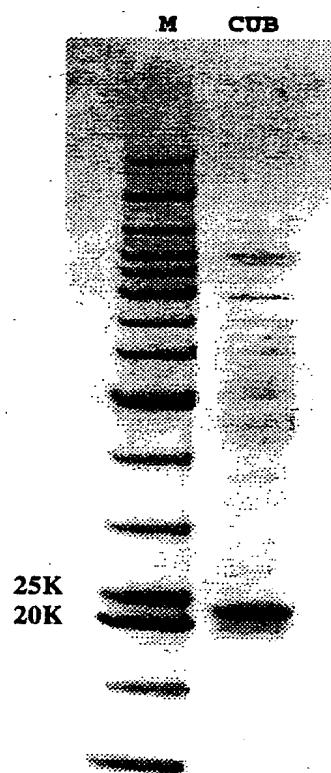


FIG. 28. The Effect of Truncated VEGF-X (CUB domain) on HUVEC Proliferation.

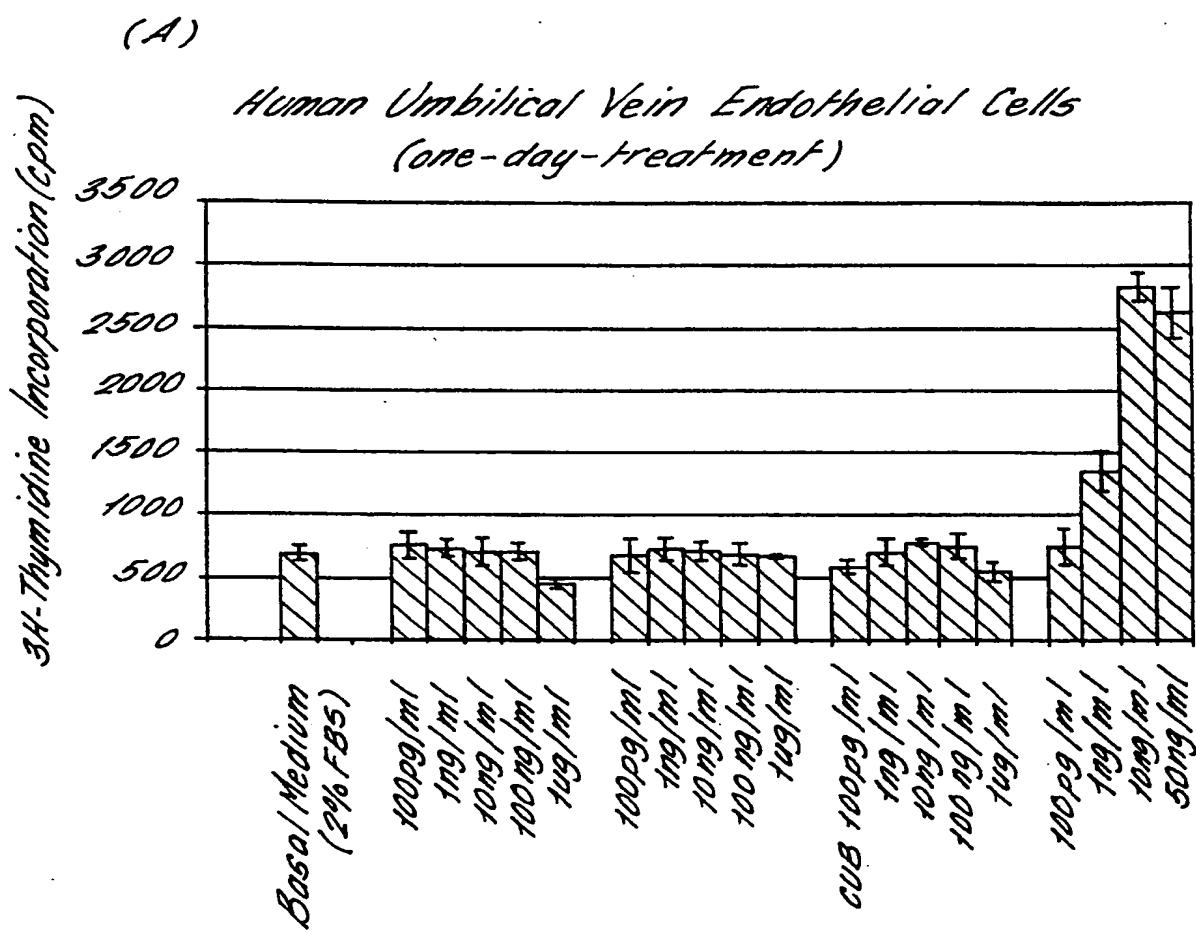


FIG. 28 (CONTINUED 1).

(B)

Human Umbilical Vein Endothelial Cells (24-hour-starving Followed by one-day-treatment)

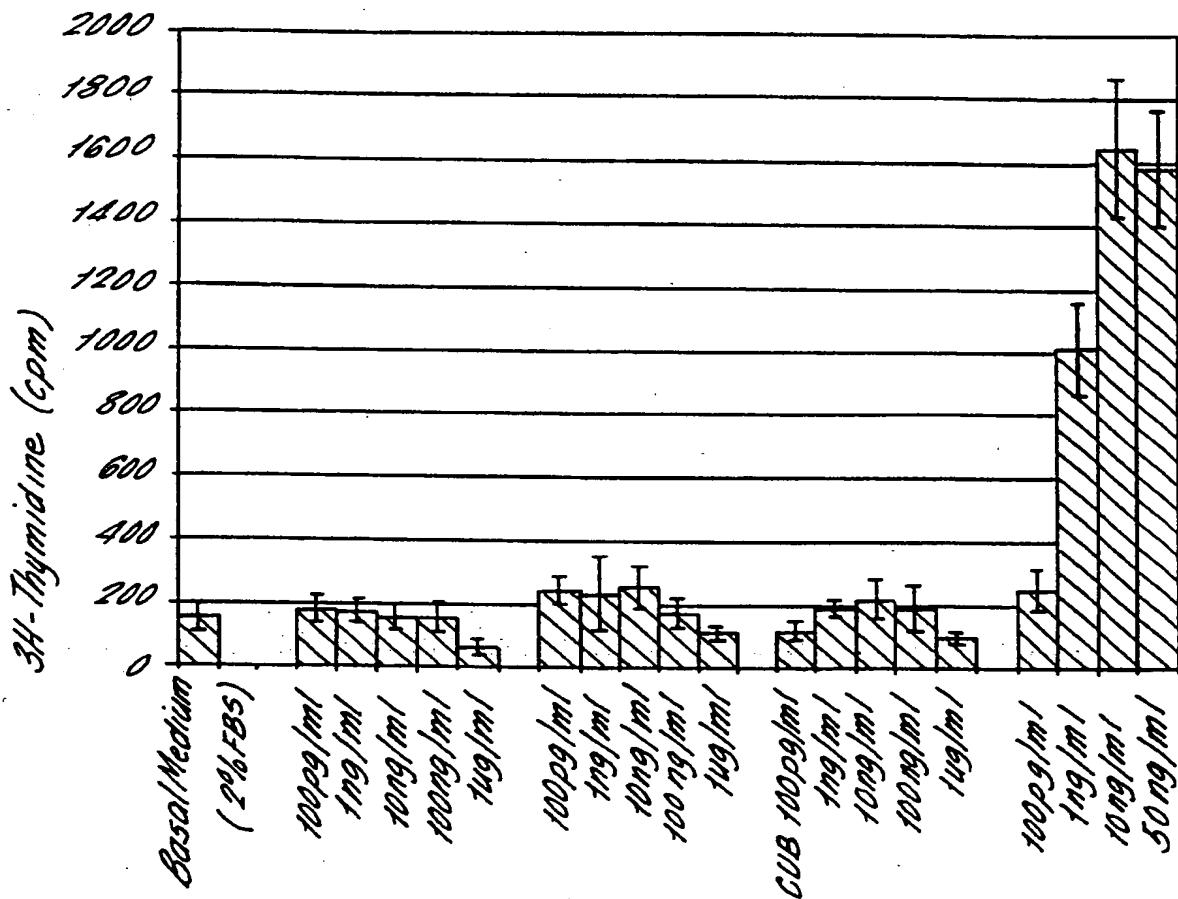


FIG. 28 (CONTINUED 2).

(C)

The effect of VEGF-A₁₆₅ and VEGF-X CUB domain on the proliferation of HUVEC (two-day-treatment).

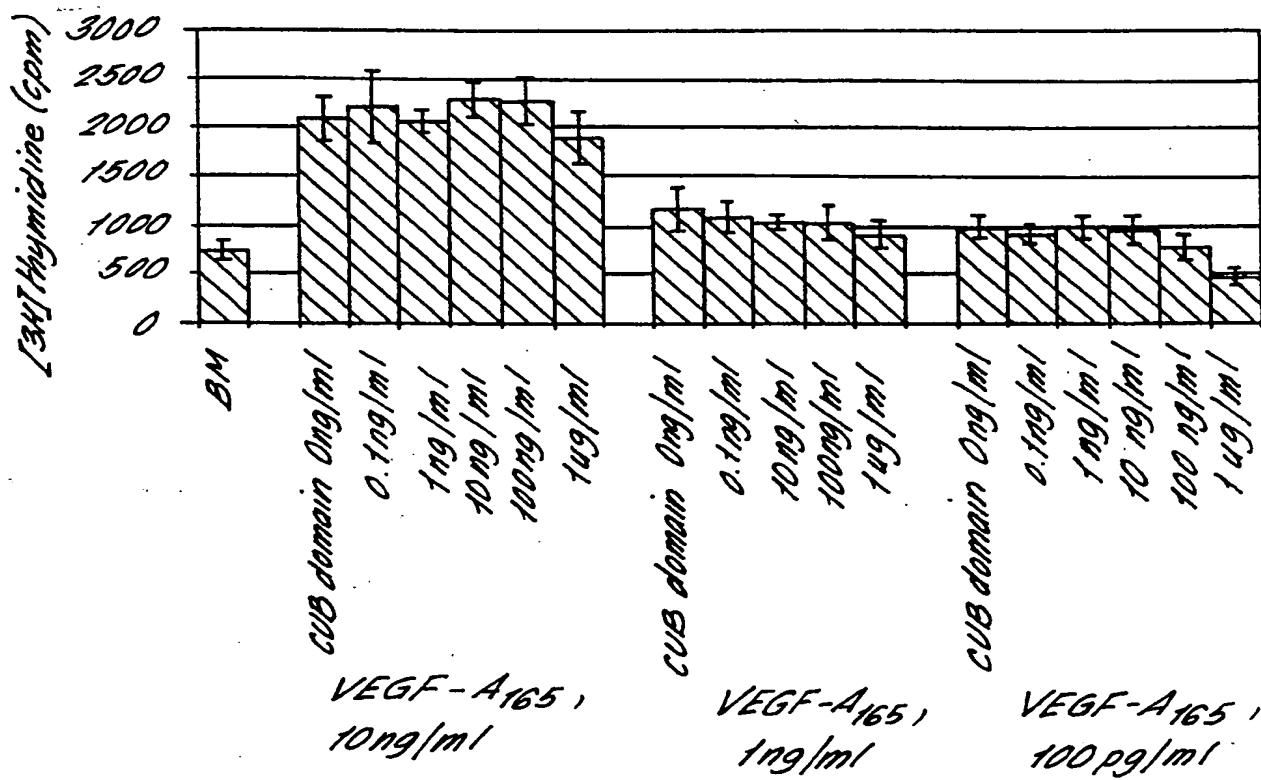
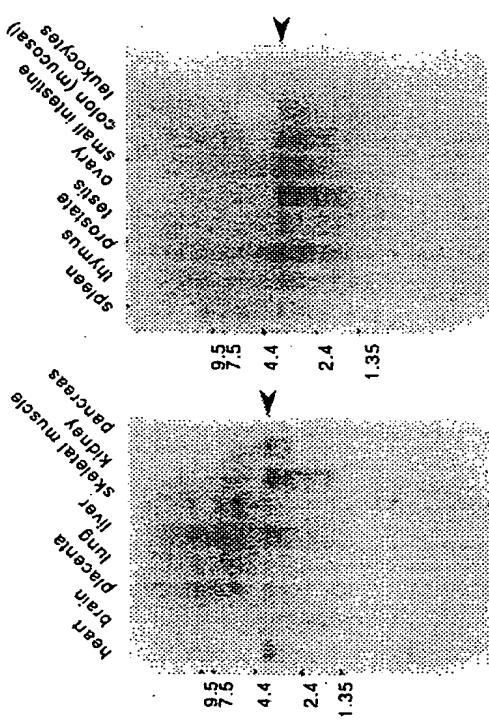


FIG. 29.

Tissue distribution of mRNA

(A) - Normal tissues



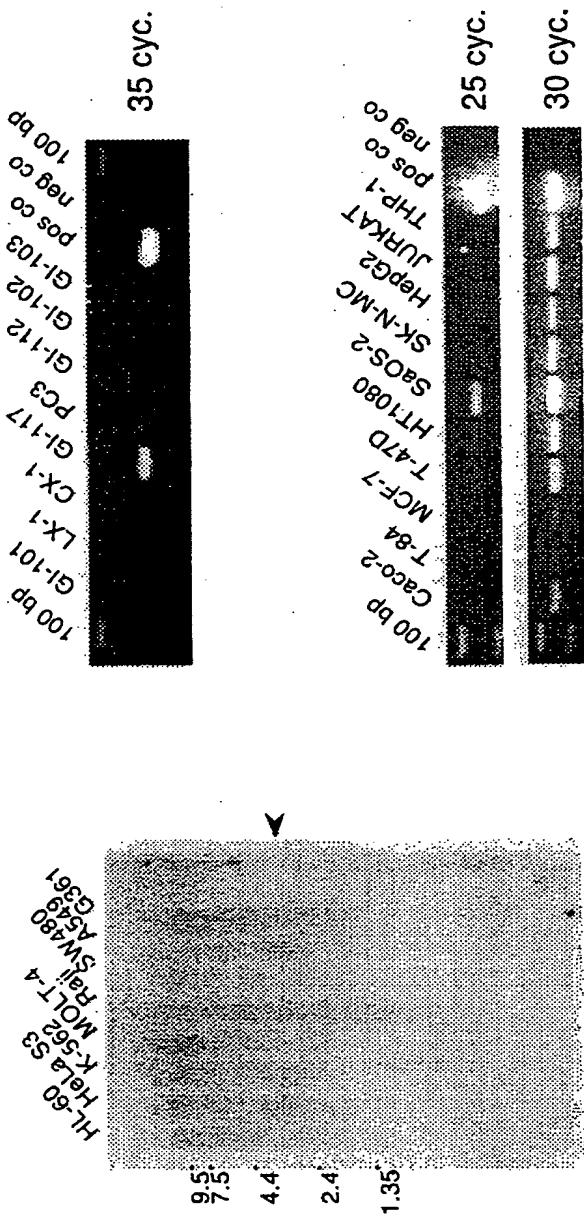


FIG. 29 (continued). (B)- Tumour tissue and cell lines

FIG. 30

Partial intron/exon structure of the VEGF-X gene

(A) - Genomic DNA sequences of 2 exons determined by sequencing

FIG. 30 (CONTINUED 1).

(B) - Location of splice sites within the cDNA sequence

1 GAATTGCCCTTTGTTAA ACCTGGGAA CTGGTCAGG TCCAGGTTT GCTTGATCC
 61 TTTCAAAAAA CTGGAGACAC AGAAGAGGGC TCTAGGAAA AGTTTGGAT GGGATTATGT
 121 GGAAACTACC CTGCGATTCT CTGCTGCCAG AGCAGGCTCG GCGCTTCCAC CCCAGTCAG
 181 CCTTCCCCTG GCGGTGGTGA AAGAGACTCG GGAGTCGCTG CTTCCAAAGT GCCCGCCGTG

+3 M S L F G L L L L T S
 241 AGTGAGCTCT CACCCCAAGTC AGCCAAATGA GCCTCTTCGG GCTTCTCCTG CTGACATCTG

+3 A L A G Q R Q G T Q A E S N L S S K F Q
 301 CCCTGGCCGG CCAGAGACAG GGGACTCAGG CGGAATCCAA CCTGAGTAGT AAATTCCAGT

+3 F S S N K E Q N G V Q D P Q H E R I I T
 361 TTTCAGCAA CAAGAACAG AACGGAGTAC AAGATCCTCA GCATGAGAGA ATTATTACTG

+3 V S T N G S I H S P R F P H T Y P R N T
 421 TGTCTACTAA TGGAAGTATT CACAGCCAA GGTTTCCCTCA TACTTATCCA AGAAATACGG

+3 V L V W R L V A V E E N V W I Q L T F D
 481 TCTTGGTATG GAGATTAGTA GCAGTAGAGG AAAATGTATG GATACTACTT ACGTTTGTG

+3 E R F G L E D P E D D I C K Y D F V E V
 541 AAAGATTTGG GCTTGAAGAC CCAGAAGATG ACATATGCAA GTATGATTT GTAGAAGTTG

+3 E E P S D G T I L G R W C G S G T V P G
 601 AGGAACCCAG TGATGAACT ATATTAGGGC GCTGGTGTGG TTCTGGTACT GTACCAAGGAA

+3 K Q I S K G N Q I R I R F V S D E Y F P
 661 AACAGATTTCAAAAGAAAT CAAATTAGGA TAAGATTTGT ATCTGATGAA TATTTCCCTT

+3 S E P G F C I H Y N I V M P Q F T E A V
 721 CTGAACCAGG GTTCTGCATC CACTACAACA TTGTCACTGCC ACAATTACACA GAACCTGTGA

+3 S P S V L P P S A L P L D L L N N A I T
 781 GTCCTTCAGT GCTACCCCT TCAGCTTGC CACTGGACCT GCTTAATAAT GCTATAACTG

+3 A F S T L E D L I R Y L E P E R W Q L D
 841 CCTTITAGTAC CTGGAGAC CTTATTCGAT ATCTGAAACC AGAGAGATGG CAGTTGGACT

+3 L E D L Y R P T W Q L L G K A F V F G R
 901 TAGAAGATCT ATATAGGCCA ACTTGGCAC ACCTGGCAA GGCTTTGTT TTGGAAAGAA

+3 K S R V V D L N L L T E E R L Y S C T
 961 AATCCAGAGT GTGGATCTG AACCTCTAA CAGAGGA~~G~~T AAGATTATAC AGCTGCACAC

+3 P R N F S V S I R E E L K R T D T I F W
 1021 CTCGTAACCTT CTCACTGTCC ATAAGGGAAAG AACTAAAGAG AACCGATAACC ATTTCTGGC

+3 P G C L L V K R C G G N C A C C L H N C
 1081 CAGGTTGTCT CCTGGTTAAA CGCTGTGGTG GGAACGTGTC CTGTTGTCTC CACAATIGCA

+3 N E C Q C V P S K V T K K Y H E V L Q L
 1141 ATGAATGTCA ATGTGTCCA AGCAAAGTTA CTAAAPATA CCACGAGGTC CTICAGTTGA

FIG. 30 (CONTINUED 2).

+3 R P K T G V R G L H K S L T D V A L E H
 1201 GACCAAAGAC CGGTGTCAGG GGATTGCACA AATCACTCAC CGACGTGGCC CTGGAGCACC

+3 H E E C D C V C R G S T G G
 1261 ATGAGGAGTG TGACTGTGTG TGCAGAGGGA GCACAGGAGG ATAGCCGCAT CACCACCGC

1321 AGCTCTGCC CAGAGCTGTG CAGTGCAGTG GCTGATTCTA TTAGAGAACG TATGCGTTAT

1381 CTCCATCCTT AAICTCAGTT GTTGCTTCA AGGAGCTTTC ATCTTCAGGA TTTACAGTGC

1441 ATTCTGAAAG AGGAGACATC AAACAGAATT AGGAGTTGTG CAACAGCTCT TTTGAGAGGA

1501 GGCCTAAAGG ACAGGAGAAA AGGTCTTCA TCGTGGAAAG AAAATTAAAT GTTGTATTAA

1561 ATAGATCACC AGCTAGTTTC AGAGTTACCA TGTACGTATT CCACTAGCTG GGTTCTGTAT

1621 TTCAGTTCTT TCGATAACGGC TTAGGGTAAT GTCAGTACAG GAAAAAAACT GTGCAAGTGA

1681 GCACCTGATT CGGTGCCTT GTTAACTCT AAAGCTCCAT GTCCTGGGCC TAAAATCGTA

1741 TAAAATCTGG ATTTTTTTT TTTTTTTTG CTCATATTCA CATATGAAA CCAGAACATT

1801 CTATGTACTA CAAACCTGGT TTTTAAAAAG GAACTATGTT GCTATGAATT AAACTTGTGT

1861 CATGCTGATA GGACAGACTG GATTTTCAT ATTTCTTATT AAAATTCTG CCATTTAGAA

1921 GAAGAGAACT ACATTCATGG TTTGGAAGAG ATAAACCTGA AAAGAAGAGT GGCCTTATCT

1981 TCACTTTATC GATAAGTCAG TTTATTGTT TCATTGTGTA CATTTTATA TTCTCCTTTT

2041 GACATTATAA CTGGGGCTT TTCTAATCTT GTTAAATATA TCTATTTTA CCAAAGGTAT

2101 TTAATATTCT TTTTATGAC AACTTAGATC AACTATTTT AGCTTGGTAA ATTTTCTAA

2161 ACACATTGT TAIAGCCAGA GGAACAAAGA TGATATAAA TATTGTTGCT CTGACAAAAA

2221 TACATGTATT TCATTCTCGT ATGGTGCTAG AGTTAGATTA ATCTGCATT TAAAAAACTG

2281 AATTGGAATA GATTGGTAA GTTGCAGA CTTTTGAAA ATAATTAAAT TATCATATCT

2341 TCCATTCTCG TTATGGAGA TGAAAATAAA AAGCAACTTA TGAAAGTAGA CATTAGATC

2401 CAGCCATTAC TAACCTATTC CTTTTTGGG GAAATCTGAG CCTAGCTCAG AAAAACATAA

2461 AGCACCTTGA AAAAGACTTG GCAGCTTCCCT GATAAAGCGT GCTGTGCTGT GCAGTAGGAA

2521 CACATCCAT TTATGTGAT GTTGTGGTTT TATTATCTTA AACTCTGTTC CATAACATTG

2581 TATAAAATACA TGGATATTTT TATGTACAGA AGTATGTCTC TTAACCAGTT CACTTATTGT

2641 ACCTGGAAGG CGGAATTCTG CAGATATC

FIG. 31.

*The Effect of F2-VEGF-X on HUVEC Proliferation:
(24-hour serum starvation followed by
one day-treatment)*

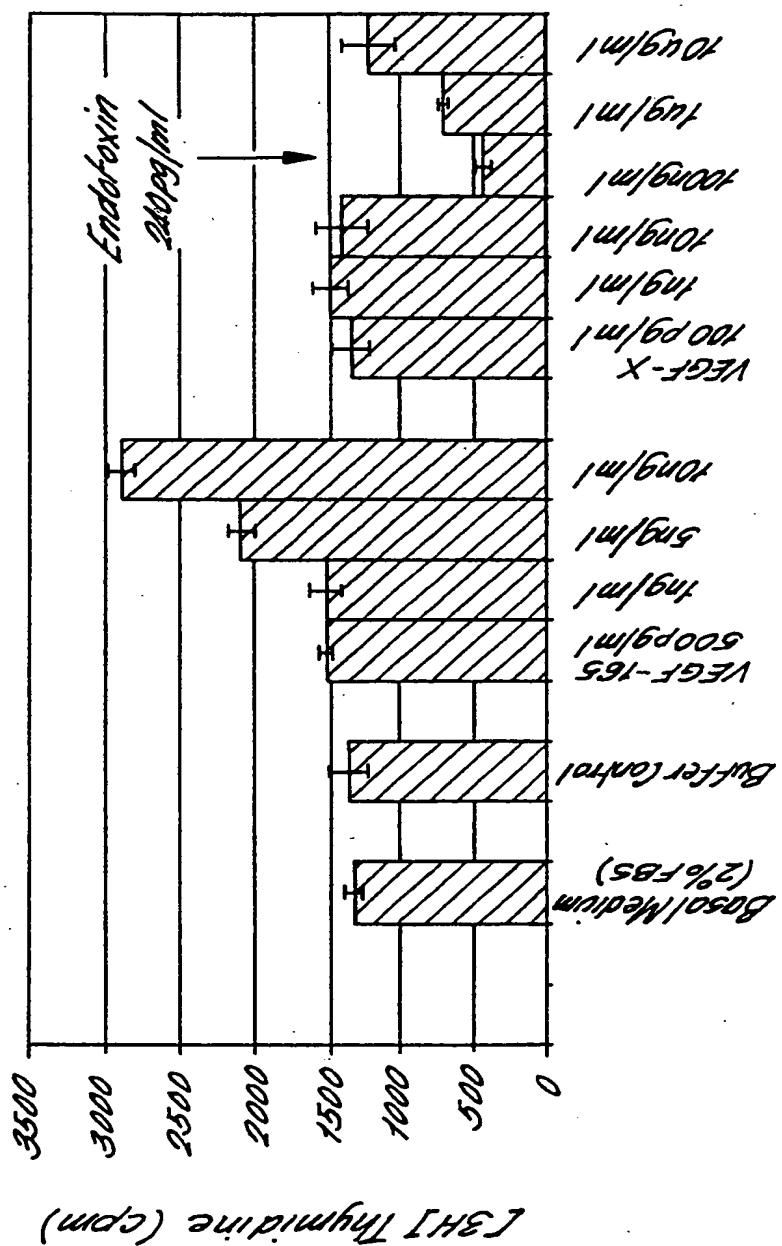


FIG. 32. The Combined Effect of Truncated VEGF-X (CVB domain) and Human Recombinant VEGF₁₆₅ on HUVEC Proliferation: (20-hour serum starvation followed by two-day-treatment)

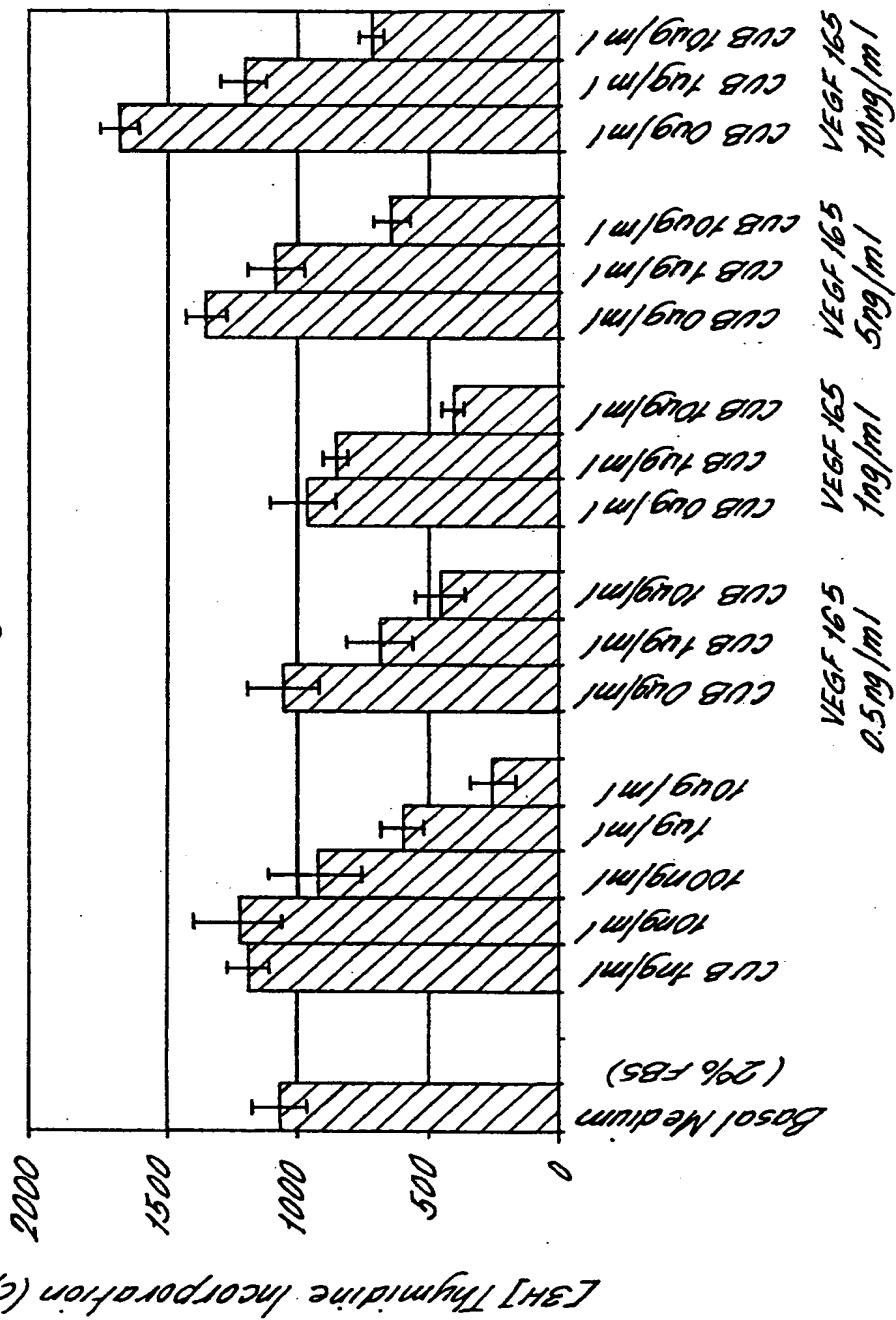
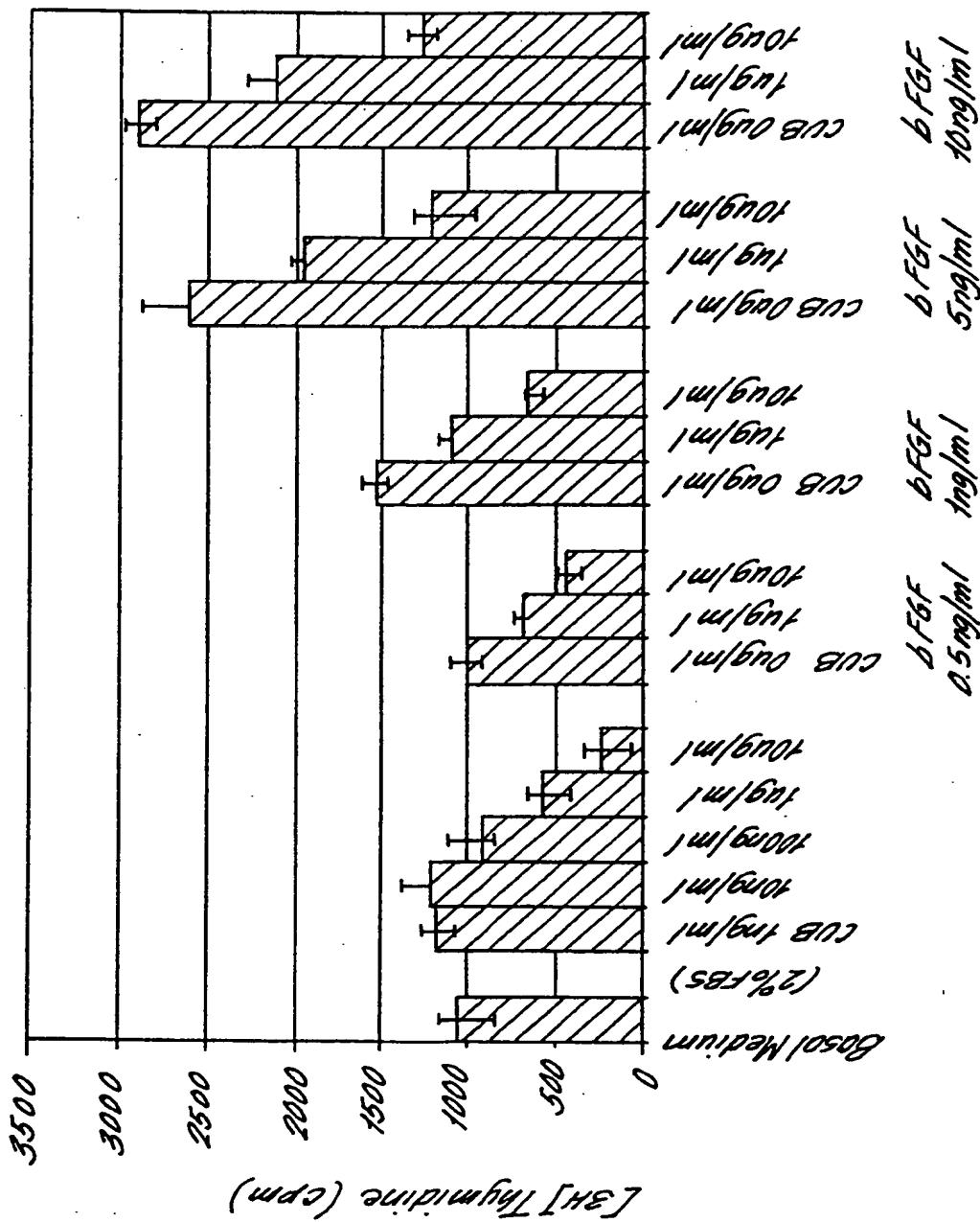


FIG. 33.
The Combined Effect of CUB Domain and Human Recombinant bFGF on HUVEC Proliferation: (24-hour serum starvation followed by two-day-treatment).



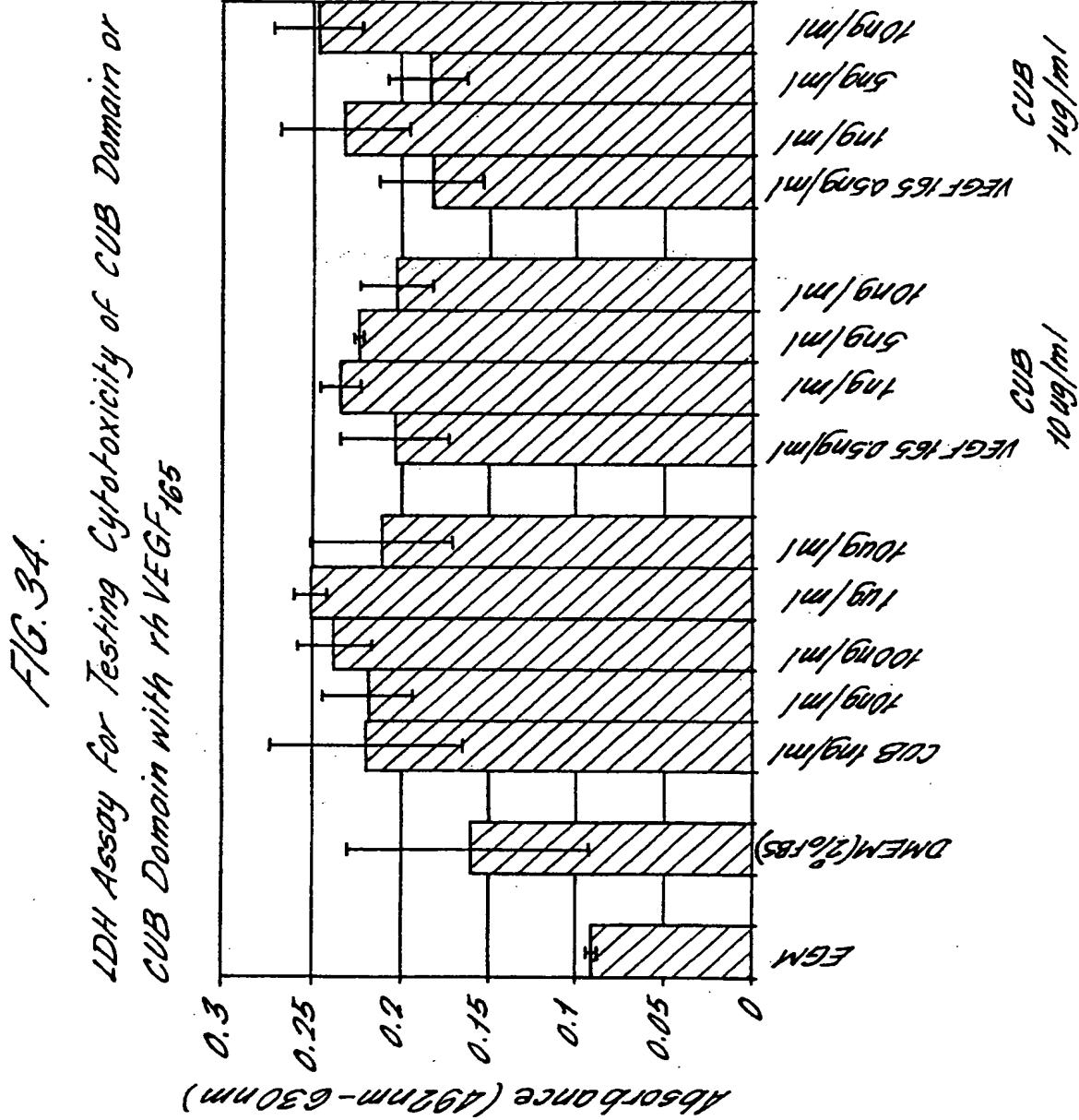
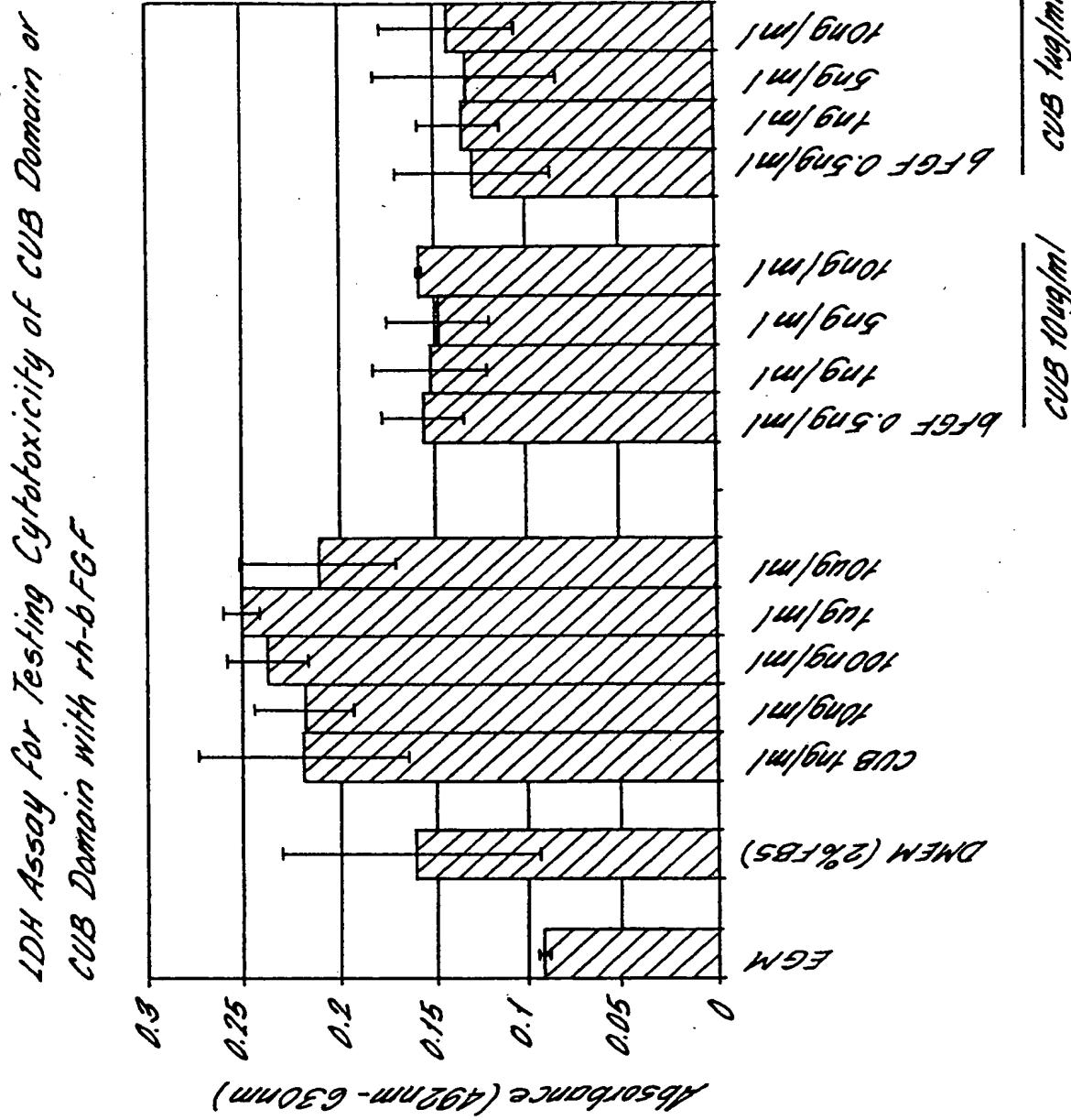


FIG. 35.



Applicant's or agent's file reference B0192/7011WO	International application No. PCT/US99/30503
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**INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>21</u> line <u>15-16</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS (BCCM)™ LABORATORIUM VOOR MOLECULAIRE BIOLOGIE - PLASMIDENCOLLECTIE (LMBP)	
Address of depositary institution (<i>including postal code and country</i>) Universiteit Gent K.L. Ledeganckstraat 35 B-9000 Gent, Belgium	
Date of deposit 20 December 1999 (20.12.99)	Accession Number LMBP 3991
C. ADDITIONAL INDICATIONS (<i>leave blank if not applicable</i>) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (<i>if the indications are not for all designated States</i>)	
E. SEPARATE FURNISHING OF INDICATIONS (<i>leave blank if not applicable</i>)	
The indications listed below will be submitted to the International Bureau later (<i>specify the general nature of the indications e.g. "Accession Number of Deposit"</i>)	

For receiving Office use only	
<input type="checkbox"/> This sheet was received with the international application	
Authorized officer	
For International Bureau use only	
<input checked="" type="checkbox"/> This sheet was received by the International Bureau on: 19 APRIL 2000 (19.04.00)	
Authorized officer Ellen Moyse	

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM™

LMBP-COLLECTION

Page 1 of Form BCCM™/LMBP/BP/4/99-23 Receipt in the case of an original deposit

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depository Authority BCCM™/LMBP identified at the bottom of next page

International Form BCCM™/LMBP/BP/4/99-23

To : Name of the depositor : Janssen Pharmaceutica N.V.

Address : Turnhoutseweg 30
B-2340 Beerse
Belgium

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

VEGF-X CUB PET22b

I.2 Accession number given by the International Depository Authority:

LMBP 3991

**BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM™
LMBP-COLLECTION**

Page 2 of Form BCCM™/LMBP/BP/4/99-23 Receipt in the case of an original deposit

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by:

(mark with a cross the applicable box(es))

- | | | |
|------------------------------------|---|--|
| — a scientific description | yes <input checked="" type="checkbox"/> | no <input type="checkbox"/> |
| — a proposed taxonomic designation | yes <input type="checkbox"/> | no <input checked="" type="checkbox"/> |

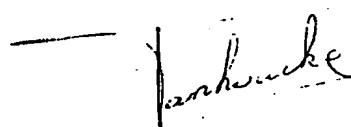
III. Receipt and acceptance

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit) : December 20, 1999

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM™)
Laboratorium voor Moleculaire Biologie - Plasmidencollectie (LMBP)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Date : January 12, 2000

Martine Vanhoucke
BCCM/LMBP curator

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM™
LMBP-COLLECTION
Page 1 of Form BCCM™/LMBP/BP/9/99-23 Viability statement

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure

Viability statement issued pursuant to Rule 10.2 by the International Depositary Authority BCCM™/LMBP identified on the following page

International Form BCCM™/LMBP/BP/9/99-23

To : Party to whom the viability statement is issued:

Name : Dr Filip De Corte

Address : Janssen Pharmaceutica N.V.
Turnhoutseweg 30
B-2340 Beerse
Belgium

I. Depositor:

I.1 Name : Janssen Pharmaceutica N.V.

I.2 Address : Turnhoutseweg 30
B-2340 Beerse
Belgium

II. Identification of the microorganism:

II.1 Accession number given by the International Depositary Authority:

LMBP 3991

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date) : December 20, 1999

III. Viability statement.

The viability of the microorganism identified under II above was tested on

: January 11, 2000

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)

viable

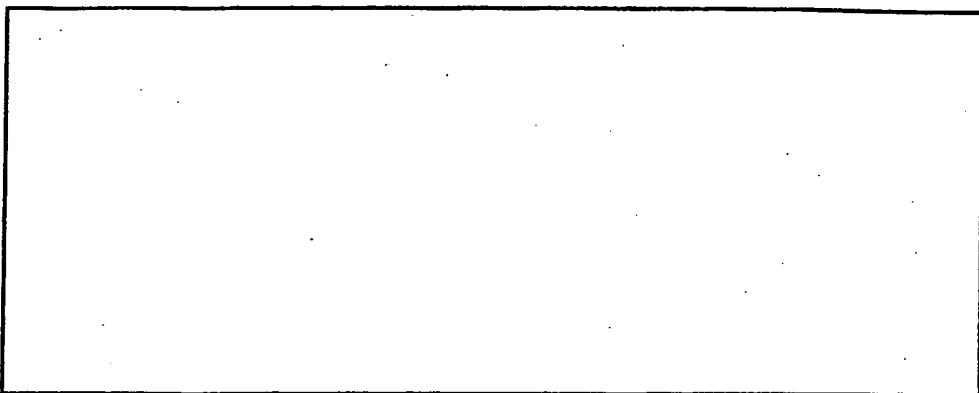
no longer viable

**BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM™
LMBP-COLLECTION**

Page 2 of Form BCCM™/LMBP/BP/9/99-23 Viability statement

IV. Conditions under which the viability test has been performed:

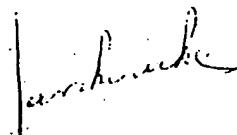
(Fill in if the information has been requested and if the results of the test were negative).



V. International Depository Authority

**Belgian Coordinated Collections of Microorganisms (BCCM™)
Laboratorium voor Moleculaire Biologie - Plasmidencollectie (LMBP)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium**

Signature(s) of person(s) having the power to represent the International Depository Authority or of authorized official(s):



Date : January 12, 2000

Martine Vanhoucke
BCCM/LMBP curator